09/834271

(FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005

```
L1        377498 S BACILLUS
L2             0 S ALPHA(A)AMYOLASE
L3         52121 S ALPHA(A)AMYLASE
L4         10118 S L1 AND L3
L5            77 S "AMYQ"
L6            57 S L4 AND L5
L7            23 DUP REM L6 (34 DUPLICATES REMOVED)
L8           310 S "TTGACA"
L9             1 S L7 AND L8
L10          428 S "TATAAT"
L11          109 S L8 AND L10
L12           35 S L1 AND L11
L13           15 DUP REM L12 (20 DUPLICATES REMOVED)
L14           26 S L5 AND PROMOTER
L15           13 DUP REM L14 (13 DUPLICATES REMOVED)
                 E WIDNER W/AU
L16          113 S E3-E9
                 E SLOMA A/AU
L17          203 S E3-E7
                 E THOMAS M D/AU
L18          518 S E3-E8
L19          795 S L16 OR L17 OR L18
L20            5 S L12 AND L19
L21            4 DUP REM L20 (1 DUPLICATE REMOVED)

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NEWS	9	MAR 03	MEDLINE file segment of TOXCENTER reloaded
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=> s bacillus
L1      377498 BACILLUS

=> s alpha(a)amyolaae
<------User Break------>

SEARCH ENDED BY USER

=> s alpha(a)amyolase
L2           0 ALPHA(A) AMYOLASE

=> s alpha(a)amylase
L3       52121 ALPHA(A) AMYLASE

=> s l1 and l3
L4       10118 L1 AND L3

=> s "amyQ"
L5          77 "AMYQ"

=> s l4 and l5
L6          57 L4 AND L5

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7           23 DUP REM L6 (34 DUPLICATES REMOVED)

=> d 1-23 ibib ab
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L7 ANSWER 1 OF 23 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005219242 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15856219
TITLE: Transcriptome analysis of the secretion stress response of **Bacillus** subtilis.
AUTHOR: Hyyrylainen Hanne-Leena; Sarvas Matti; Kontinen Vesa P
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute (KTL), Helsinki, Finland,. Vesa.Kontinen@ktl.fi
SOURCE: Applied microbiology and biotechnology, (2005 May) 67 (3) 389-96. Electronic Publication: 2005-01-27.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050429
Last Updated on STN: 20050429

AB Transcription profiling of all protein-encoding genes of **Bacillus** subtilis was carried out under several secretion stress conditions in the exponential growth phase. Cells that secreted **AmyQ** **alpha-amylase** at a high level were stressed only moderately: seven genes were induced, most significantly htrA and htrB, encoding quality control proteases, and yqxL, encoding a putative CorA-type Mg(2+) transporter. These three genes were induced more strongly by severe secretion stress (prsA3 mutant secreting **AmyQ**), suggesting that their expression responds to protein misfolding. In addition, 17 other genes were induced, including the liaIHGFSR (yvqIHGFEC) operon, csaA and ffh, encoding chaperones involved in the pretranslocational phase of secretion, and genes involved in cell wall synthesis/modification. Severe secretion stress caused downregulation of 23 genes, including the prsA paralogue yacD. Analysis of a cssS knockout mutant indicated that the absence of the CssRS two-component system, and consequently the absence of the HtrA and HtrB proteases, caused secretion stress. The results also suggest that the htrA and htrB genes comprise the CssRS regulon. B. subtilis cells respond to secretion/folding stress by various changes in gene expression, which can be seen as an attempt to combat the stress condition.

L7 ANSWER 2 OF 23 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:807461 SCISEARCH
THE GENUINE ARTICLE: 851CD
TITLE: Subcellular sites for bacterial protein export
AUTHOR: Campo N; Tjalsma H; Buist G; Stepniak D; Meijer M; Veenhuis M; Westermann M; Muller J P; Bron S; Kok J; Kuipers O P (Reprint); Jongbloed J D H
CORPORATE SOURCE: Univ Groningen, Groningen Biomol Sci & Biotechnol Inst, Dept Genet, Kerklaan 30, NL-9751 NN Haren, Netherlands (Reprint); Univ Groningen, Groningen Biomol Sci & Biotechnol Inst, Dept Genet, NL-9751 NN Haren, Netherlands; Groningen Biomol Sci & Biotechnol Inst, NL-9750 AA Haren, Netherlands; Univ Jena Klinikum, Elektronenmikroskop Zentrum, D-07743 Jena, Germany; Univ Jena, Inst Mol Biol, D-07745 Jena, Germany
COUNTRY OF AUTHOR: Netherlands; Germany
SOURCE: MOLECULAR MICROBIOLOGY, (SEP 2004) Vol. 53, No. 6, pp. 1583-1599.
Publisher: BLACKWELL PUBLISHING LTD, 9600 GARSINGTON RD, OXFORD OX4 2DG, OXON, ENGLAND.
ISSN: 0950-382X.
DOCUMENT TYPE: Article; Journal

LANGUAGE: English
REFERENCE COUNT: 43
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Most bacterial proteins destined to leave the cytoplasm are exported to extracellular compartments or imported into the cytoplasmic membrane via the highly conserved SecA-YEG pathway. In the present studies, the subcellular distributions of core components of this pathway, SecA and SecY, and of the secretory protein pre-**AmyQ**, were analysed using green fluorescent protein fusions, immunostaining and/or immunogold labelling techniques. It is shown that SecA, SecY and (pre-)**AmyQ** are located at specific sites near and/or in the cytoplasmic membrane of **Bacillus** subtilis. The localization patterns of these proteins suggest that the Sec machinery is organized in spiral-like structures along the cell, with most of the translocases organized in specific clusters along these structures. However, this localization appears to be independent of the helicoidal structures formed by the actin-like cytoskeletal proteins, MreB or Mbl. Interestingly, the specific localization of SecA is dynamic, and depends on active translation. Moreover, reducing the phosphatidylglycerol phospholipids content in the bacterial membrane results in delocalization of SecA, suggesting the involvement of membrane phospholipids in the localization process. These data show for the first time that, in contrast to the recently reported uni-ExPortal site in the coccoid Streptococcus pyogenes, multiple sites dedicated to protein export are present in the cytoplasmic membrane of rod-shaped B. subtilis.

L7 ANSWER 3 OF 23 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]

AB DERWENT ABSTRACT:
NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis **alpha-amylase** gene (amyE), B. licheniformis **alpha-amylase** gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene

(penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens **alpha-amylase** gene (**amyQ**). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L7 ANSWER 4 OF 23 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-22251 BIOTECHDS
TITLE: Modulating Sec-dependent protein secretion, comprises introducing a spoIIIJ or yqjG gene linked to an inducible promoter into a **Bacillus** cell and modulating the expression of the spoIIIJ or yqjG gene; vector-mediated gene transfer and expression in host cell for strain improvement
AUTHOR: BRON S; TJALSMA H; VAN DIJL J M
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003060068 24 Jul 2003
APPLICATION INFO: WO 2002-US39634 12 Dec 2002
PRIORITY INFO: US 2002-426832 15 Nov 2002; US 2002-348080 9 Jan 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-598514 [56]
AB DERWENT ABSTRACT:

NOVELTY - Modulating Sec-dependent protein secretion comprising introducing a spoIIIJ or yqjG gene linked to an inducible promoter into a **Bacillus** cell, and modulating the expression of the spoIIIJ or yqjG gene by varying the level of induction of the inducible promoter, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a

purified DNA molecule comprising an inducible promoter operatively linked to the spoIIIJ or yqjG gene; and (2) a method of modulating the secretion of a protein of interest comprising forming a first DNA molecule encoding a chimeric protein comprising a Sec-dependent secretion signal peptide, forming a second DNA molecule encoding an inducible promoter operably linked to the spoIIIJ or yqjG gene, transforming a host cell with the DNA molecule, and growing the host cell under conditions where the protein of interest is expressed at the desired level.

WIDER DISCLOSURE - Methods of inhibiting sporulation in a **Bacillus** cell comprising a mutation of the spoIIIJ gene, where the mutation results in the formation of an inactive gene product, are also disclosed.

BIOTECHNOLOGY - Preferred Method: Alternatively, modulating Sec-dependent protein secretion comprises providing a **Bacillus** cell comprising spoIIIJ and yqjG genes linked to an endogenous high expression promoter, and modulating the expression of the spoIIIJ and yqjG genes by varying the level of induction of the promoter. The (inducible) promoter is the Pspac promoter. In modulating the secretion of a protein of interest, the host cell is grown under conditions where the inducible promoter is induced. The protein of interest is expressed at low level.

USE - The methods are useful for enhancing the secretion of proteins from a host cell, preferably from a **Bacillus** cell, that may be made to be secreted via the Sec-dependent secretion pathway. The DNA molecules are useful for the inducible expression of the spoIIIJ and/or yqjG genes.

EXAMPLE - To evaluate the importance of yqjG and spoIIIJ function for protein secretion, **Bacillus** subtilis DELTAyqjG, DELTAspoIIIJ and DELTAyqjG-IspoIIIJ, as well as the parental strain 168 were transformed with plasmid pLip2031 for the secretion of the B. subtilis lipase LipA, pPSPPhoA5 for the secretion of the alkaline phosphatase PhoA of Escherichia coli fused to the prepro-region of the lipase gene from Staphylococcus hyicus, or pKTH10 for the secretion of the **alpha-amylase AmyQ**. In order to deplete B. subtilis DELTAyqjG-IspoIIIJ of spoIIIJ, this strain was grown for 3 hours in tryptone/yeast extract (TY) medium without isopropyl-beta-D-thiogalacto-pyranoside (IPTG). As a control, TY medium with 50 nM IPTG or 500 nM IPTG was used. The secretion of LipA, PhoA and **AmyQ** was analyzed by Western blotting. The levels of LipA, PhoA and **AmyQ** in the medium of spoIIIJ-depleted cells of B. subtilis DELTAyqjG-IspoIIIJ (no IPTG) were significantly reduce compared to those in the media of the fully induced double mutant (500 nM IPTG), or the parental strain 168. The levels of the LipA and PhoA in the media of DELTAyqjG-IspoIIIJ strains that were fully induced with IPTG (500 nM) were higher than those in the media of the parental control strains. This suggests that over expression of the spoIIIJ gene can result in improved protein secretion in B. subtilis.(50 pages)

L7 ANSWER 5 OF 23 MEDLINE on STN DUPLICATE 3

ACCESSION NUMBER: 2003237859 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12606539

TITLE: Production of **Bacillus** anthracis protective antigen is dependent on the extracellular chaperone, PrsA.

AUTHOR: Williams Rachel C; Rees Mark L; Jacobs Myra F; Pragai Zoltan; Thwaite Joanne E; Baillie Leslie W J; Emmerson Peter T; Harwood Colin R

CORPORATE SOURCE: School of Cell and Molecular Biosciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, United Kingdom.

SOURCE: Journal of biological chemistry, (2003 May 16) 278 (20) 18056-62. Electronic Publication: 2003-02-26. Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030523
Last Updated on STN: 20030626
Entered Medline: 20030625

AB Protective antigen (PA) is a component of the **Bacillus** anthracis lethal and edema toxins and the basis of the current anthrax vaccine. In its heptameric form, PA targets host cells and internalizes the enzymatically active components of the toxins, namely lethal and edema factors. PA and other toxin components are secreted from B. anthracis using the Sec-dependent secretion pathway. This requires them to be translocated across the cytoplasmic membrane in an unfolded state and then to be folded into their native configurations on the trans side of the membrane, prior to their release from the environment of the cell wall. In this study we show that recombinant PA (rPA) requires the extracellular chaperone PrsA for efficient folding when produced in the heterologous host, B. subtilis; increasing the concentration of PrsA leads to an increase in rPA production. To determine the likelihood of PrsA being required for PA production in its native host, we have analyzed the B. anthracis genome sequence for the presence of genes encoding homologues of B. subtilis PrsA. We identified three putative B. anthracis PrsA proteins (PrsAA, PrsAB, and PrsAC) that are able to complement the activity of B. subtilis PrsA with respect to cell viability and rPA secretion, as well as that of **AmyQ**, a protein previously shown to be PrsA-dependent.

L7 ANSWER 6 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:72937 HCAPLUS
DOCUMENT NUMBER: 140:265284
TITLE: Genome engineering reveals large dispensable regions in **Bacillus** subtilis
AUTHOR(S): Westers, Helga; Dorenbos, Ronald; van Dijl, Jan Maarten; Kabel, Jorrit; Flanagan, Tony; Devine, Kevin M.; Jude, Florence; Seror, Simone J.; Beekman, Aaeron C.; Darmon, Elise; Eschevins, Caroline; de Jong, Anne; Bron, Sierd; Kuipers, Oscar P.; Albertini, Alessandra M.; Antelmann, Haike; Hecker, Michael; Zamboni, Nicola; Sauer, Uwe; Bruand, Claude; Ehrlich, Dusko S.; Alonso, Juan C.; Salas, Margarita; Quax, Wim J.
CORPORATE SOURCE: Department of Pharmaceutical Biology, University of Groningen, Groningen, Neth.
SOURCE: Molecular Biology and Evolution (2003), 20(12), 2076-2090
CODEN: MBEVEO; ISSN: 0737-4038
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bacterial genomes contain 250 to 500 essential genes, as suggested by single gene disruptions and theor. considerations. If this view is correct, the remaining nonessential genes of an organism, such as **Bacillus** subtilis, have been acquired during evolution in its perpetually changing ecol. niches. Notably, .apprx.47% of the .apprx.4100 genes of B. subtilis belong to paralogous gene families in which several members have overlapping functions. Thus, essential gene functions will outnumber essential genes. To answer the question to what extent the most recently acquired DNA contributes to the life of B. subtilis under standard laboratory growth conditions, the authors initiated a "reconstruction" of the B. subtilis genome by removing prophages and AT-rich islands. Stepwise deletion of two prophages (SPβ, PBSX), three prophage-like regions, and the largest operon of B. subtilis (pks) resulted in a genome reduction of

7.7% and elimination of 332 genes. The resulting strain was phenotypically characterized by metabolic flux anal., proteomics, and specific assays for protein secretion, competence development, sporulation, and cell motility. Thus, genome engineering is a feasible strategy for functional anal. of large gene clusters, and that removal of dispensable genomic regions may pave the way toward an optimized **Bacillus** cell factory.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 23 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003120704 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12634326
TITLE: The extracytoplasmic folding factor PrsA is required for protein secretion only in the presence of the cell wall in **Bacillus** subtilis.
AUTHOR: Wahlstrom Eva; Vitikainen Marika; Kontinen Vesa P; Sarvas Matti
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, Mannerheimintie 166, FIN-00300, Helsinki, Finland.
SOURCE: Microbiology (Reading, England), (2003 Mar) 149 (Pt 3) 569-77.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030314
Last Updated on STN: 20030611
Entered Medline: 20030610

AB Pulse-chase labelling was used to study the role of the cell wall microenvironment in the functioning of **Bacillus** subtilis PrsA, an extracellular lipoprotein and member of the parvulin family of peptidylprolyl cis/trans-isomerases. It was found that in protoplasts, and thus in the absence of a cell wall matrix, the post-translocational folding, stability and secretion of the **AmyQ alpha-amylase** were independent of PrsA, in contrast to the strict dependency found in rods. The results indicate that PrsA is dedicated to assisting the folding and stability of exported proteins in the particular microenvironment of the cytoplasmic membrane-cell wall interface, possibly as a chaperone preventing unproductive interactions with the wall. The data also provide evidence for a crucial role of the wall in protein secretion. The presence of the wall directly or indirectly facilitates the release of **AmyQ** from the cell membrane and affects the rate of the signal peptide processing.

L7 ANSWER 8 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:457872 HCAPLUS
DOCUMENT NUMBER: 139:163626
TITLE: Production of Chlamydia pneumoniae proteins in **Bacillus** subtilis and their use in characterizing immune responses in the experimental infection model
AUTHOR(S): Airaksinen, Ulla; Penttila, Tuula; Wahlstrom, Eva; Vuola, Jenni M.; Puolakkainen, Mirja; Sarvas, Matti
CORPORATE SOURCE: Department of Vaccines, National Public Health Institute, Helsinki, Finland
SOURCE: Clinical and Diagnostic Laboratory Immunology (2003), 10(3), 367-375
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal
LANGUAGE: English
AB Due to intracellular growth requirements, large-scale cultures of chlamydiae and purification of its proteins are difficult and laborious. To overcome these problems we produced chlamydial proteins in a heterologous host, **Bacillus** subtilis, a gram-pos. nonpathogenic bacterium. The genes of Chlamydia pneumoniae major outer membrane protein (MOMP), the cysteine-rich outer membrane protein (Omp2), and the heat shock protein (Hsp60) were amplified by PCR, and the PCR products were cloned into expression vectors containing a promoter, a ribosome binding site, and a truncated signal sequence of the .**alpha**.-**amylase** gene from **Bacillus** amyloliquefaciens. C. pneumoniae genes were readily expressed in B. subtilis under the control of the .**alpha** .-**amylase** promoter. The recombinant proteins MOMP and Hsp60 were purified from the bacterial lysate with the aid of the carboxy-terminal histidine hexamer tag by affinity chromatog. The Omp2 was separated as an insol. fraction after 8 M urea treatment. The purified proteins were successfully used as immunogens and as antigens in serol. assays and in a lymphoproliferation test. The Omp2 and Hsp60 antigens were readily recognized by the antibodies appearing after pulmonary infection following intranasal inoculation of C. pneumoniae in mice. Also, splenocytes collected from mice immunized with MOMP or Hsp60 proteins proliferated in response to in vitro stimulation with the corresponding proteins.
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 23 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002100306 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11807061
TITLE: ClpXP protease regulates the signal peptide cleavage of secretory preproteins in **Bacillus** subtilis with a mechanism distinct from that of the Ecs ABC transporter.
AUTHOR: Pummi Tiina; Leskela Soile; Wahlstrom Eva; Gerth Ulf; Tjalsma Harold; Hecker Michael; Sarvas Matti; Kontinen Vesa P
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, FIN-00300 Helsinki, Finland.
SOURCE: Journal of bacteriology, (2002 Feb) 184 (4) 1010-8. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020208
Last Updated on STN: 20030403
Entered Medline: 20020319
AB Identification and characterization of a suppressor mutation, sup-15, which partially restored secretion in the protein secretion-deficient **Bacillus** subtilis ecsA26 mutant, led us to discover a novel function of Clp protease. Inactivation of ClpP improved the processing of the precursor of **AmyQ alpha-amylase** exposed on the outer surface of the cytoplasmic membrane. A similar improvement of **AmyQ** secretion was conferred by inactivation of the ClpX substrate-binding component of the ClpXP complex. In the absence of ClpXP, the transcription of the sipS, sipT, sipV, and lsp signal peptidase genes was elevated two- to fivefold, a likely cause of the improvement of the processing and secretion of **AmyQ** and complementation of ecs mutations. Specific overproduction of SipT enhanced the secretion. These findings extend the regulatory roles of ClpXP to protein secretion. ClpXP also influenced the processing of the lipoprotein PrsA. A concerted regulation of signal peptidase genes by a ClpXP-dependent activator is

suggested. In contrast, Ecs did not affect transcription of the sip genes, pointing to a different mechanism of secretion regulation.

L7 ANSWER 10 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:161441 HCAPLUS
DOCUMENT NUMBER: 134:190018
TITLE: .**alpha**.-**Amylase** variants with improved detergent performance
INVENTOR(S): Svendsen, Allan; Kjaerulff, Soeren; Bisgaard-Frantzen, Henrik; Andersen, Carsten
PATENT ASSIGNEE(S): Novo-Nordisk A/S, Den.; Novo Alle
SOURCE: U.S., 36 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6197565	B1	20010306	US 1998-193068	19981116
US 6887986	B1	20050503	US 1999-441313	19991116
PRIORITY APPLN. INFO.:			US 1998-193068 A2	19981116

AB The invention relates to a variant of a parent Termamyl-like .**alpha**.-**amylase**, comprising mutations in two, three, four, five or six regions/positions. The variants have increased stability at high temps. (relative to the parent). The variants comprise addnl. mutations added to the LE174 hybrid α-enzyme in which the 35 N-terminal residues of **Bacillus** licheniformis .**alpha**.-**amylase** are replaced by residues 1-33 of BAN/B. amyloliquefaciens .**alpha**.-**amylase**. The invention also relates to a DNA construct comprising a DNA sequence encoding an .**alpha**.-**amylase** variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .**alpha**.-**amylase** variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .**alpha**.-**amylase** variant of the invention, a manual or automatic dishwashing detergent composition comprising an .**alpha**.-**amylase** variant of the invention, a method for generating a variant of a parent Termamyl-like .**alpha**.-**amylase**, which variant exhibits increased.
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 11 OF 23 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2001196320 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11222585
TITLE: Quantitation of the capacity of the secretion apparatus and requirement for PrsA in growth and secretion of **alpha-amylase** in **Bacillus** subtilis.
AUTHOR: Vitikainen M; Pummi T; Airaksinen U; Wahlstrom E; Wu H; Sarvas M; Kontinen V P
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, FIN-00300 Helsinki, Finland.
SOURCE: Journal of bacteriology, (2001 Mar) 183 (6) 1881-90.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20010410
Entered Medline: 20010405

AB Regulated expression of **AmyQ alpha-amylase** of **Bacillus** amyloliquefaciens was used to examine the capacity of the protein secretion apparatus of B. subtilis. One B. subtilis cell was found to secrete maximally 10 fg of **AmyQ** per h. The signal peptidase SipT limits the rate of processing of the signal peptide. Another limit is set by PrsA lipoprotein. The wild-type level of PrsA was found to be 2 x 10(4) molecules per cell. Decreasing the cellular level of PrsA did not decrease the capacity of the protein translocation or signal peptide processing steps but dramatically affected secretion in a posttranslocational step. There was a linear correlation between the number of cellular PrsA molecules and the number of secreted **AmyQ** molecules over a wide range of prsA and **amyQ** expression levels. Significantly, even when **amyQ** was expressed at low levels, overproduction of PrsA enhanced its secretion. The finding is consistent with a reversible interaction between PrsA and **AmyQ**. The high cellular level of PrsA suggests a chaperone-like function. PrsA was also found to be essential for the viability of B. subtilis. Drastic depletion of PrsA resulted in altered cellular morphology and ultimately in cell death.

L7 ANSWER 12 OF 23 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2001684194 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11555295
TITLE: A novel two-component regulatory system in **Bacillus** subtilis for the survival of severe secretion stress.
AUTHOR: Hyyrylainen H L; Bolhuis A; Darmon E; Muukkonen L; Koski P; Vitikainen M; Sarvas M; Pragai Z; Bron S; van Dijl J M; Kontinen V P
CORPORATE SOURCE: Laboratory of Vaccine Development, National Public Health Institute, FIN-00300, Helsinki, Finland.
SOURCE: Molecular microbiology, (2001 Sep) 41 (5) 1159-72. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011204
Last Updated on STN: 20020212
Entered Medline: 20020211

AB The Gram-positive eubacterium **Bacillus** subtilis is well known for its high capacity to secrete proteins into the environment. Even though high-level secretion of proteins is an efficient process, it imposes stress on the cell. The present studies were aimed at the identification of systems required to combat this so-called secretion stress. A two-component regulatory system, named CssR-CssS, was identified, which bears resemblance to the CpxR-CpxA system of Escherichia coli. The results show that the CssR/S system is required for the cell to survive the severe secretion stress caused by a combination of high-level production of the **alpha-amylase AmyQ** and reduced levels of the extracytoplasmic folding factor PrsA. As shown with a prsA3 mutation, the Css system is required to degrade misfolded exported proteins at the membrane-cell wall interface. This view is supported by the observation that transcription of the htrA gene, encoding a predicted membrane-bound protease of B. subtilis, is strictly controlled by CssS. Notably, CssS represents the first identified sensor for extracytoplasmic protein misfolding in a Gram-positive eubacterium. In conclusion, the results show that quality control systems for extracytoplasmic protein folding are not exclusively present in the periplasm of Gram-negative

eubacteria, but also in the Gram-positive cell envelope.

L7 ANSWER 13 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:133032 HCAPLUS
DOCUMENT NUMBER: 137:334696
TITLE: Screening for mutants defective in secretion
AUTHOR(S): Koski, Pertti; Sarvas, Matti
CORPORATE SOURCE: National Public Health Institute, Helsinki, Finland
SOURCE: Functional Analysis of Bacterial Genes (2001), 143-148. Editor(s): Schumann, Wolfgang; Ehrlich, S. Dusko; Ogasawara, Naotake. John Wiley & Sons Ltd.: Chichester, UK.
CODEN: 69CHC2; ISBN: 0-471-49008-3
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review describes the plate halo tests for screening secretion mutants. Protocols for quantitating **Bacillus** subtilis .**alpha**.-**amylase** and lichenase accumulated in culture medium are also presented. The plate halo test is applicable only when the B. subtilis strain to be tested is engineered for an elevated level of .**alpha**.-**amylase**. The pulse-chase procedure is applicable for B. subtilis strains expressing **AmyQ** .**alpha**.-**amylase** expressed from the **amyQ** gene in the plasmid pKTH10 or from a single copy of **amyQ** in the chromosome.

L7 ANSWER 14 OF 23 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2000472614 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10871614
TITLE: D-Alanine substitution of teichoic acids as a modulator of protein folding and stability at the cytoplasmic membrane/cell wall interface of **Bacillus** subtilis.
AUTHOR: Hyyrylainen H L; Vitikainen M; Thwaite J; Wu H; Sarvas M; Harwood C R; Kontinen V P; Stephenson K
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, FI-00300 Helsinki, Finland.
SOURCE: Journal of biological chemistry, (2000 Sep 1) 275 (35) 26696-703.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001012
Last Updated on STN: 20001012
Entered Medline: 20001003
AB The extracytoplasmic folding of secreted proteins in Gram-positive bacteria is influenced by the microenvironment of the compartment into which they are translocated, namely the negatively charged matrix of the cell wall polymers. In this compartment, the PrsA lipoprotein facilitates correct post-translocational folding or prevents misfolding of secreted proteins. In this study, a secretion mutant of B. subtilis (prsA3) encoding a defective PrsA protein was mutagenized and screened for restored secretion of the **AmyQ alpha-amylase**. One mini-Tn10 insertion, which partially suppressed the secretion deficiency, was found to interrupt dlt, the operon involved in the d-alanylation of teichoic acids. The inactivation of dlt rescued the mutant PrsA3 protein from degradation, and the increased amount of PrsA3 was shown to enhance the secretion of PrsA-dependent proteins. Heterologous or abnormal secreted proteins, which are prone to degradation after translocation, were also stabilized and secreted in increased quantities from a dlt prsA(+) strain. Furthermore, the dlt mutation

partially suppressed the lethal effect of PrsA depletion, suggesting that the dlt deficiency also leads to stabilization of an essential cell wall protein(s). Our results suggest that main influence of the increased net negative charge of the wall caused by the absence of d-alanine is to increase the rate of post-translocational folding of exported proteins.

L7 ANSWER 15 OF 23 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-01575 BIOTECHDS
TITLE: Immunity to Chlamydia pneumoniae induced by vaccination with DNA vector expressing a cytoplasmic protein (Hsp60) or outer membrane proteins (MOMP and Omp2);
nucleic acid vaccine, cysteine cytoplasmic protein and outer membrane proteins useful for inducing immune response
AUTHOR: Penttila T; Vuola J M; Puurula V; Anttila M; Anttila M; Sarvas M; Rautonen N; Makela P H; Puolakkainen M
CORPORATE SOURCE: Univ.Helsinki; Nat.Public-Health-Inst.Helsinki; Nat.Vet.Food-Res.Inst.Helsinki
LOCATION: Department of Virology, POB 21, Haartman Institute ,University of Helsinki, FIN-00014 Helsinki Finland. Email: tuula.penttila@helsinki.fi
SOURCE: Vaccine; (2000) 19, 9-10, 1256-65
CODEN: VACCDE
ISSN: 0264-410X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Immunity to Chlamydia pneumoniae induced by vaccination with DNA vectors expressing a cytoplasmic protein (Hsp60) or outer membrane protein (MOMP and Omp2), was studied. Mycoplasma-free C. pneumoniae K6 was propagated in HL cell in minimal essential medium with 10% fetal cattle serum and 0.3 mg/ml L-glutamine. Recombinant C. pneumoniae protein MOMP, Omp2 and Hsp60 were produced in **Bacillus** subtilis. The momp, omp2 and Hsp60 were amplified by polymerase chain reaction, and cloned into the expression vector containing the promoter, RBS and a short 5' stretch of the **alpha-amylase** (EC-3.2.1.1) gene (**amyQ**).
C. pneumoniae genes encoding for MOMP, Omp2 and Hsp60 were cloned into an eukaryotic expression vector plasmid pcDNA3.1. Immunization with pmomp or phsp60 showed 1.2-1.5 log reduction in the mean lung bacterial counts after the challenge. Specific antibodies were detected only in sera of mice immunized with pmomp2 and phsp60. Immunization with any of the three vaccines did not reduce the severity of histologically assessed pneumonia, but resulted in significantly higher lymphoid reaction in the lung indicating immunological memory. (43 ref)

L7 ANSWER 16 OF 23 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:121629 SCISEARCH
THE GENUINE ARTICLE: 397CK
TITLE: Development of marker-free strains of **Bacillus** subtilis capable of secreting high levels of industrial enzymes
AUTHOR: Widner B (Reprint); Thomas M; Sternberg D; Lammon D; Behr R; Sloma A
CORPORATE SOURCE: Novo Nordisk Biotech Inc, Davis, CA 95616 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, (OCT 2000) Vol. 25, No. 4, pp. 204-212.
Publisher: NATURE AMERICA INC, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707 USA.
ISSN: 1367-5435.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Different strategies have been employed to achieve high-level expression of single-copy genes encoding secreted enzymes in **Bacillus** subtilis, A model system was developed which utilizes the aprL gene from **Bacillus** clausii as a reporter gene for monitoring expression levels during stationary phase. An exceptionally strong promoter was constructed by altering the nuceotide sequence in the -10 and -35 regions of the promoter for the **amyQ** gene of **Bacillus** amyloliquefaciens. In addition, two or three tandem copies of this promoter were shown to increase expression levels substantially in comparison to the monomer promoter alone. Finally, the promoter and mRNA stabilization sequences derived from the cry3A gene of **Bacillus** thuringiensis were used in combination with the mutant **amyQ** promoter to achieve the highest levels of aprL expression, These promoters were shown to be fully functional in a high-expressing **Bacillus** strain grown under industrial fermentation conditions. The ability to obtain maximum expression levels from a single copy gene now makes it feasible to construct environmentally friendly, marker-free industrial strains of B. subtilis.

L7 ANSWER 17 OF 23 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 9

ACCESSION NUMBER: 1999-15556 BIOTECHDS
TITLE: Production of polypeptide in **Bacillus** sp. using specific promoters, particularly for producing enzymes; the effect of a short consensus **amyQ** promoter on recombinant **alpha-amylase** production via vector-mediated gene transfer and expression in **Bacillus** subtilis
AUTHOR: Widner W; Sloma A; Thomas M D
PATENT ASSIGNEE: Novo-Nordisk-Biotech
LOCATION: Davis, CA, USA.
PATENT INFO: WO 9943835 2 Sep 1999
APPLICATION INFO: WO 1999-US4360 26 Feb 1999
PRIORITY INFO: US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-561370 [47]

AB The production of a protein (I) in **Bacillus** sp. cells using specific tandem or consensus promoters is new. Also claimed are: the production of the recombinant **Bacillus** sp. cells via the introduction of a nucleic acid construct; the production of **Bacillus** sp. mutants which contain no selectable marker genes by treating the cells to delete a marker gene; marker-free mutant cell produced using this method; isolated consensus **alpha-amylase** (**amyQ**) promoter sequence made up of 2 185 bp DNA sequences (specified); a nucleic acid construct containing a sequence (II), which encodes (I), linked to one or more copies of the **amyQ** promoter; and a recombinant vector and **Bacillus** sp. cells containing this construct. This new method may be useful for producing homologs or particularly heterologous proteins, particularly enzymes (specifically serine protease, maltogenic **alpha-amylase** , EC-3.2.1.1 and pullulanase, EC-3.2.1.41), but also hormones, antibodies, reporters, etc. In an example, the replacement of the **amyQ** promoter with a short consensus **amyQ** promoter resulted in a increase in enzyme expression of 620% in **Bacillus** subtilis strain PL801 cells. (89pp)

L7 ANSWER 18 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:311291 HCAPLUS
DOCUMENT NUMBER: 130:334680
TITLE: .**alpha**.-**Amylase** mutants with improved wash performance

INVENTOR(S): Borchert, Torben Vedel; Svendsen, Allan; Andersen, Carsten; Nielsen, Bjarne Ronfeld; Nissen, Torben Lauesgaard; Kjaerulff, Soren
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9923211	A1	19990514	WO 1998-DK471	19981030
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2308119	AA	19990514	CA 1998-2308119	19981030
AU 9897373	A1	19990524	AU 1998-97373	19981030
EP 1027428	A1	20000816	EP 1998-951291	19981030
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
BR 9813328	A	20000822	BR 1998-13328	19981030
US 6204232	B1	20010320	US 1998-183412	19981030
JP 2001521739	T2	20011113	JP 2000-519071	19981030
US 2001039253	A1	20011108	US 2001-769864	20010125
US 6673589	B2	20040106		
US 2004038368	A1	20040226	US 2003-665667	20030919
US 2005084937	A1	20050421	US 2004-980923	20041104

PRIORITY APPLN. INFO.:

DK 1997-1240	A	19971030
DK 1998-936	A	19980714
US 1997-64662P	P	19971106
US 1998-93234P	P	19980717
US 1998-183412	A3	19981030
WO 1998-DK471	W	19981030
US 2001-769864	A3	20010125
US 2003-665667	B1	20030919

AB The invention relates to a variant of a parent Termamyl-like **alpha**.-**amylase**, which exhibits an alteration in at least one of the following properties relative to said parent **alpha**.-**amylase**: (i) improved pH stability at a pH from 8 to 10.5; and/or (ii) improved Ca2+ stability at pH 8 to 10.5, and/or (iii) increased specific activity at temps. from 10 to 60°. Thus, variants were prepared from wild-type **alpha**.-**amylases** from **Bacillus** strain NCIB 12512, Kasamyl (**Bacillus** strain NCIB 12513), Termamyl (**Bacillus** licheniformis), and B. amyloliquefaciens.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 19 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:77694 HCAPLUS
DOCUMENT NUMBER: 130:134974
TITLE: Characterization of the **Bacillus** subtilis secretion factor SecDF and use in enhanced production and secretion of desired heterologous or homologous proteins
INVENTOR(S): Quax, Wilhelmus J.

PATENT ASSIGNEE(S): Genencor International, Inc., USA; Genencor International B.V.
SOURCE: PCT Int. Appl., 54 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9904007	A1	19990128	WO 1998-US14786	19980716
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2296689	AA	19990128	CA 1998-2296689	19980715
EP 1003873	A1	20000531	EP 1998-935747	19980715
EP 1003873	B1	20050406		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
AU 9884931	A1	19990210	AU 1998-84931	19980716
JP 2001510047	T2	20010731	JP 2000-503213	19980716
US 6258563	B1	20010710	US 2000-462844	20000322
US 2002006641	A1	20020117	US 2001-899482	20010705
US 6630328	B2	20031007		

PRIORITY APPLN. INFO.:
EP 1997-305286 A 19970716
EP 1997-305344 A 19970717
WO 1998-US14786 W 19980716
US 2000-462844 A1 20000322

AB The present invention provides expression vectors, methods and systems for enhanced production and secretion of desired heterologous or homologous proteins in gram-pos. microorganisms using the **Bacillus** subtilis secretion factor SecDF. The present invention provided the nucleic acid and amino acid sequences for the B. subtilis secretion factor SecDF. The B. subtilis secretion factor SecDF, in contrast to the SecD and SecF of Escherichia coli, was found to be encoded by one nucleic acid sequence (gene secDF). The protein sequence of B. subtilis secretion factor SecDF was found to be identical to the protein sequence found in GenBank Accession AF024506. The membrane topol. of B. subtilis secretion factor SecDF was described and SecDF was shown to be required for efficient secretion of **AmyQ**.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 20 OF 23 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1999157560 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10027970
TITLE: Ecs, an ABC transporter of **Bacillus** subtilis: dual signal transduction functions affecting expression of secreted proteins as well as their secretion.
AUTHOR: Leskela S; Wahlstrom E; Hyyrylainen H L; Jacobs M; Palva A; Sarvas M; Kontinen V P
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, Helsinki, Finland.
SOURCE: Molecular microbiology, (1999 Jan) 31 (2) 533-43.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429

AB ecs is a three-cistron operon of **Bacillus** subtilis, encoding proteins with similarity to the ATPase (EcsA) and hydrophobic components (EcsB) of ABC transporters. The ecsA26 point mutation was shown to cause a strong processing defect of a secreted **alpha-amylase** precursor (preAmyQ) and of three other exoproteins. Northern analysis of the level of **amyQ** mRNA showed that ecsA26 also decreases **amyQ** transcription. This effect too was pleiotropic, as judged by a drastic decrease in the expression from an exoprotease promoter of a reporter protein. A knockout mutation of the ecsB cistron caused a processing defect similar to ecsA26 but, unlike ecsA26, did not affect **amyQ** transcription. These was also no defect in transcription in the ecsA ecsB double mutant. Thus, an intact ecsB product was required for the downregulation of **amyQ** by the mutant ecsA. These results suggest a dual regulatory function for Ecs, in which Ecs, possibly as part of a signal transduction mechanism, regulates some component(s) of the protein secretion apparatus as well as secretory protein transcription in a co-ordinated fashion.

L7 ANSWER 21 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:352949 HCAPLUS
DOCUMENT NUMBER: 129:27099
TITLE: Methods for producing polypeptides in surfactin mutants of **Bacillus** cells
INVENTOR(S): Sloma, Alan; Sternberg, David; Adams, Lee F.; Brown, Stephen
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9822598	A1	19980528	WO 1997-US21084	19971118
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9854450	A1	19980610	AU 1998-54450	19971118
EP 941349	A1	19990915	EP 1997-948365	19971118
EP 941349	B1	20030730		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
CN 1240482	A	20000105	CN 1997-180644	19971118
JP 2001503641	T2	20010321	JP 1998-523825	19971118
AT 246251	E	20030815	AT 1997-948365	19971118

PRIORITY APPLN. INFO.:	US 1996-749521	A	19961118
	US 1997-49441P	P	19970612
	US 1996-749421	A	19961118
	WO 1997-US21084	W	19971118

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a mutant of a **Bacillus** cell, wherein the mutant (i) comprises a first nucleic acid sequence encoding the polypeptide and a second nucleic acid sequence comprising a modification

of at least one of the genes responsible for the biosynthesis or secretion of a surfactin or isoform thereof under conditions conducive for the production of the polypeptide and (ii) the mutant produces less of the surfactin or isoform thereof than the **Bacillus** cell when cultured under the same conditions; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to mutants of **Bacillus** cells and methods for producing the mutants. B. subtilis ΔspoIIAc ΔnprE ΔaprE ΔamyE ΔsrfC strains were prepared and transformed with an **amyQ** promoter-amyM chimeric gene. Culture of these strains resulted in less foaming and resultant volume loss than culture of strains containing the srfC gene.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 22 OF 23 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1995:742740 HCAPLUS
DOCUMENT NUMBER: 123:173579
TITLE: Variants of .**alpha**.-**amylase** of **Bacillus**, preparation of variants, and their improved activity as washing detergents
INVENTOR(S): Bisgaard-Frantzen, Henrik; Borchert, Torben Vedel; Svendsen, Allan; Thellersen, Marianne; Van Der Zee, Pia
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 105 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9510603	A1	19950420	WO 1994-DK370	19941005
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2173329	AA	19950420	CA 1994-2173329	19941005
AU 9478074	A1	19950504	AU 1994-78074	19941005
EP 722490	A1	19960724	EP 1994-928775	19941005
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
CN 1134725	A	19961030	CN 1994-194081	19941005
BR 9407767	A	19970318	BR 1994-7767	19941005
JP 09503916	T2	19970422	JP 1994-511196	19941005
FI 9601524	A	19960530	FI 1996-1524	19960404
PRIORITY APPLN. INFO.:			DK 1993-1133 A	19931008
			DK 1994-140 A	19940202
			WO 1994-DK370 W	19941005

AB A variant of a parent .**alpha**.-**amylase** enzyme having an improved washing and/or dishwashing performance as compared to the parent enzyme, wherein one or more amino acid residues of the parent enzyme have been replaced by a different amino acid residue and/or wherein one or more amino acid residues of the parent .**alpha**.-**amylase** have been deleted and/or wherein one or more amino acid residues have been added to the parent .**alpha**.-**amylase** enzyme, provided that the variant is different from one in which the methionine residue in position 197 of a parent B. licheniformis .**alpha**.-**amylase** has been replaced by alanine or threonine, as the only modification being made. The variant may be used for washing and dishwashing.

L7 ANSWER 23 OF 23 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-01709 BIOTECHDS
TITLE: Cloning and expression of an amylase gene from **Bacillus** stearothermophilus; thermostable **alpha-amylase** expression in **Bacillus** subtilis and **Bacillus** licheniformis (conference paper)
AUTHOR: Diderichsen B; Poulsen G B; Jorgensen P L
CORPORATE SOURCE: Novo-Nordisk
LOCATION: Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark.
SOURCE: Res.Microbiol.; (1991) 142, 7-8, 793-96
CODEN: RMCREW
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **Bacillus** stearothermophilus **alpha-amylase** (EC-3.2.1.1) gene, amyS, was cloned and expressed in **Bacillus** subtilis under its own expression signals. The AmyS yield was 200-fold higher than in the B. stearothermophilus donor. However, compared to other **alpha-amylases** cloned in B. subtilis, yields were low. Yields were increased 4-fold by the insertion of 2 promoters (Pm and Pq from the amyM gene of B. stearothermophilus and **Bacillus** amyloliquefaciens, respectively) in tandem, upstream of the amyS promoter. A suitable plasmid harboring amyS transcribed by the amyM and **amyQ** promoters was introduced by protoplast transformation into a **Bacillus** licheniformis strain that expressed negligible amounts of AmyL. The resulting strain showed a 3-fold increase in AmyS productivity compared to an equivalent B. subtilis construction. Replacement of the amyS promoter, ribosome binding site and signal peptide with the corresponding functions from amyL did not increase yields further. Thermostable **alpha-amylase** is used for the industrial production of glucose or high fructose syrups. (12 ref)

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=> s "TTGACA"
L8          310 "TTGACA"

=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1       377498 S BACILLUS
L2            0 S ALPHA(A)AMYOLASE
L3        52121 S ALPHA(A)AMYLASE
L4        10118 S L1 AND L3
L5           77 S "AMYQ"
L6           57 S L4 AND L5
L7           23 DUP REM L6 (34 DUPLICATES REMOVED)
L8          310 S "TTGACA"

=> s l7 and l8
L9            1 L7 AND L8

=> d all

L9    ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN    2004-08483  BIOTECHDS
TI    Production of a secreted polypeptide having L-asparaginase activity for
      treating leukemia, comprises cultivating a host cell comprising a nucleic
      acid having a sequence encoding a secretory signal peptide linked to a
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second sequence;
vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy

AU THOMAS M D; SLOMA A
PA NOVOZYMES BIOTECH INC
PI US 2003186380 2 Oct 2003
AI US 2003-406025 1 Apr 2003
PRAI US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DT Patent
LA English
OS WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis **alpha-amylase** gene (amyE), B. licheniformis **alpha-amylase** gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens **alpha-amylase** gene (**amyQ**). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

CC THERAPEUTICS, Protein Therapeutics; GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; OTHER CHEMICALS, Miscellaneous Chemicals; BIOMANUFACTURING and BIOCATALYSIS, Biocatalyst Isolation and Characterization; BIOMANUFACTURING and BIOCATALYSIS, Biocatalyst Application

CT RECOMBINANT SECRETED L-ASPARAGINASE PREP., VECTOR-MEDIATED SECRETORY SIGNAL PEPTIDE, **BACILLUS** SP. TANDEM PROMOTER GENE TRANSFER, EXPRESSION IN **BACILLUS** SUBTILIS, **BACILLUS** ALKALOPHILUS, **BACILLUS** AMYLOLIQIFACIENS, **BACILLUS** BREVIS, **BACILLUS** CIRCULANS, **BACILLUS** CLAUSSI, **BACILLUS** COAGULANS, **BACILLUS** LAUTUS, **BACILLUS** LENTUS, **BACILLUS** LICHENIFORMIS, **BACILLUS** MEGATERIUM, **BACILLUS** STEAROTHERMOPHILUS, **BACILLUS** THURINGIENSIS, L-ASPARAGINE CONVERSION, APPL. L-ASPARTIC ACID PREP., ACUTE LYMPHOCYTIC LEUKEMIA THERAPY ENZYME CYTOSTATIC EC-3.5.1.1 BACTERIUM AMINO ACID CANCER (23, 17)

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=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1        377498 S BACILLUS
L2             0 S ALPHA(A)AMYOLASE
L3         52121 S ALPHA(A)AMYLASE
L4         10118 S L1 AND L3
L5            77 S "AMYQ"
L6            57 S L4 AND L5
L7            23 DUP REM L6 (34 DUPLICATES REMOVED)
L8           310 S "TTGACA"
L9             1 S L7 AND L8

=> s "TATAAT"
L10          428 "TATAAT"

=> s l8 and l10
L11          109 L8 AND L10

=> s l1 and l11
L12           35 L1 AND L11

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           15 DUP REM L12 (20 DUPLICATES REMOVED)

=> d 1-15 ibib ab

L13  ANSWER 1 OF 15  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2004:903415  HCAPLUS
DOCUMENT NUMBER:          142:234066
TITLE:                    Cloning and characterization of gene promoters from
                          Bacillus pumilus
AUTHOR(S):                Pan, Jiao; Zhang, Yizheng
CORPORATE SOURCE:         Sichuan Key Laboratory of Molecular Biology and
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Biotechnology, College of Life Science, Sichuan University, Chengdu, 610064, Peop. Rep. China
SOURCE: High Technology Letters (2004), 10(2), 17-20
CODEN: HTLEFC; ISSN: 1006-6748
PUBLISHER: High Technology Letters Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB DNA fragments obtained from Sau3AI partially digested total DNA of **Bacillus** pumilus UN31-C-42 are first inserted into BamHI site of pSUPV4, a promoter-probe vector. The recombinant DNA mols. are transformed into Escherichia coli cells and eight-three Kanr clones (named pSUBp1-pSUBp83) are obtained. The inserted fragments in pSUBp53, pSUBp57, pSUBp21, which showed high level of kanamycin - resistance, are sequenced and analyzed, resp. These fragments contain some conserved sequences of prokaryotic gene promoters, such as **TATAAT** and **TTGACA** box. The promoter fragment Bp53 could efficiently promote the alkaline protease gene of B. pumilus expression not only in E. coli but also in B. subtilis cells.
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 2 OF 15 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:
NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome

of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, amyQ, amyM, cryIIIA, dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L13 ANSWER 3 OF 15 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-00390 BIOTECHDS
TITLE: Creating a library of artificial promoters comprises mixing oligonucleotides in a polymerase chain reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters; artificial protein library construction and vector expression in host cell for use in gene expression level determination
AUTHOR: SOUCAILLE P
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003089605 30 Oct 2003
APPLICATION INFO: WO 2003-US12045 18 Apr 2003
PRIORITY INFO: US 2002-374627 22 Apr 2002; US 2002-374627 22 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-854112 [79]
AB DERWENT ABSTRACT:

NOVELTY - Creating a library of artificial promoters comprises mixing a first oligonucleotide and a second oligonucleotide in an amplification reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters.

DETAILED DESCRIPTION - The above method comprises: (a) obtaining an insertion DNA cassette comprising a first recombinase site, a second

recombinase site and a selective marker gene located between the first and the second recombinase sites; (b) obtaining a first oligonucleotide comprising a first nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest, and a second nucleic acid fragment homologous to a 5' end of the insertion DNA cassette; (c) obtaining a second oligonucleotide comprising (i) a third nucleic acid fragment homologous to a 3' end of the insertion DNA cassette, (ii) a precursor promoter comprising a -35 consensus region (-35 to -30), a linker sequence and a -10 consensus region (-2 to -7), where the linker sequence comprises 4-20 nucleotides and is flanked by the -35 region and the -10 region, where the precursor promoter has been modified to include at least one modified nucleotide position of the promoter and where the -35 region and the -10 region each include 4-6 conserved nucleotides of the promoter, and (iii) a fourth nucleic acid fragment homologous to a downstream region of the transcription start site of the promoter; and (d) mixing the first oligonucleotide and the second oligonucleotide in an amplification reaction with the insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters. INDEPENDENT CLAIMS are also included for the following: (1) an artificial promoter library comprising a mixture of double-stranded polynucleotides which include, in sequential order: a nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest; a first recombinase site; a nucleic acid sequence encoding an antimicrobial resistance gene; a second recombinase gene; 2 consensus regions of a promoter and a linker sequence, where the first consensus region comprises the -35 region and the second region comprises the -10 region cited above; and a nucleic acid fragment homologous to the downstream region of the +1 transcription start site of the promoter; (2) methods of modifying a promoter in selected host cells; (3) a method of creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest; and (4) transformed bacterial cells selected from the method in (3).

BIOTECHNOLOGY - Preferred Method: Creating a library of artificial promoters further comprises purifying the amplified products. The amplification step is a polymerase chain reaction (PCR) step. The -35 region of the precursor promoter is selected from **TTGACA**, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA and TTCAAA. It comprises a modification to the -30 residue of the promoter. The -10 region is selected from TAAGAT, **TATAAT**, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT. Preferably, the -35 region is **TTGACA** and the -10 region is **TATAAT** or AATAAT. The linker sequence comprises 16-18 nucleotides. The precursor promoter is obtained from a promoter selected from Ptrc, PD/E20, PH207, PN25, PG25, PJ5, PA1, PA2, PA3, Plac, PGI, PlacUV5, PCON, and Pbls. Each of the precursor promoters comprises a sequence fully defined in the specification. The library of artificial promoters includes 3 sequences of 60 bp each fully defined in the specification. The precursor promoter and the chromosomal gene of interest are homologous or heterologous. The method further comprises modifying the ribosome binding site, including: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest; a modified ribosome binding site of the gene of interest, the binding site includes at least one modified nucleotide; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified ribosome binding sites. The ribosome binding site from the precursor promoter is selected from any of the 27 nucleotide sequences (e.g. AGGAAA, AGAAAA or AGAAGA) fully defined in the specification. The method further comprises inserting a stabilizing mRNA sequence between the modified ribosome binding site and a transcription initiation site of the third oligonucleotide, and altering the start codon of the gene of interest in the third oligonucleotide.

Alternatively, the method comprises: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest; a start codon of the gene of interest, where the start codon is degenerated and includes at least one modification; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified start codons. It also comprises inserting a stabilizing mRNA sequence between the -10 box of the artificial promoter and a transcription initiation site of the third oligonucleotide. Modifying a promoter in selected host cells comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR library, where the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination; growing the transformed bacterial cells; and selecting the transformed bacterial cells comprising the artificial promoters. The bacterial host cell is selected from Escherichia coli, Pantoea sp. and **Bacillus** sp.. Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR products, where the PCR products comprising the artificial promoters are integrated into bacterial host cells by homologous recombination to produce transformed bacterial cells; growing the transformed bacteria cells; and obtaining a library of transformed bacterial cells where the library exhibits a range of expression levels of a chromosomal gene of interest. The method further comprises selecting transformed bacterial cells from the library. The selected transformed bacterial cells have a low or high level of expression of the gene of interest. The method also comprises excising the selective marker gene from the transformed bacterial cells. Preferred Promoter Library: The double-stranded polynucleotides further include a modified ribosome binding site of the promoter, a modified start codon or a stabilizing mRNA nucleic acid sequence, where the binding site, start codon or mRNA sequence is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site. The -35 region includes a substitution in one nucleotide position with the remaining nucleotide positions conserved. The promoter library further includes a substitution in one nucleotide position of the -10 region with the remaining nucleotide positions conserved.

USE - The method is useful in creating a library of bacterial clones with varying levels of gene expression. The method is used in developing a quick and efficient means of determining the optimum expression level of a gene in a metabolic pathway which, in turn, results in an optimization of strain performance for a desired product.

ADVANTAGE - A direct advantage of the method is that a bacterial clone may be selected based on the expression level obtained from the DNA libraries and then be ready for use in a fermentation process where cell viability is not negatively affected by expression of the gene of interest. (44 pages)

L13 ANSWER 4 OF 15 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-00389 BIOTECHDS
TITLE: Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprises transforming bacterial host cells with a promoter library that comprises at least two promoter cassettes; promoter library construction and vector expression in host cell for use in gene expression level determination
AUTHOR: CERVIN M A; VALLE F
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003089604 30 Oct 2003

APPLICATION INFO: WO 2003-US12044 18 Apr 2003
PRIORITY INFO: US 2002-374735 22 Apr 2002; US 2002-374627 22 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-854111 [79]
AB DERWENT ABSTRACT:

NOVELTY - Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprising transforming bacterial host cells with a promoter library that comprises at least two promoter cassettes, is new.

DETAILED DESCRIPTION - Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprising: (a) obtaining a promoter library comprising at least two promoter cassettes; (b) transforming bacterial host cells with the promoter library, where the promoter cassettes are integrated into the bacterial host cells by homologous recombination to produce transformed host cells; (c) culturing the transformed host cells under suitable growth conditions; and (d) obtaining a library of transformed bacterial cells, where the transformed bacterial cells exhibit a range of expression levels of a chromosomal gene of interest, is new. INDEPENDENT CLAIMS are also included for: (1) a promoter cassette comprising in sequential order: (a) a 5' sequence homologous to an upstream flanking region of a target site; (b) a first recombinase recognition site; (c) a selectable marker; (d) a second recombinase recognition site; (e) a modified precursor promoter comprising at least one modified nucleotide in a position corresponding to a -35 consensus region, a linker sequence or a -10 consensus region of a precursor promoter; and (f) a 3' sequence homologous to a downstream flanking region of the target site; (2) a promoter library comprising at least two promoter cassettes cited above; (3) a vector comprising the promoter cassette cited above; (4) a host cell transformed with the above promoter cassette; (5) modifying the regulatory function of a native promoter of a chromosomal gene of interest, comprising obtaining the above promoter cassette, transforming a host cell with the promoter cassette to allow homologous recombination between the promoter cassette and homologous flanking regions of a target site, where the cassette replaces a native promoter region of a chromosomal gene of interest, and culturing the transformed host cells under suitable growth conditions; (6) altering the expression of a chromosomal gene of interest, comprising obtaining the above promoter cassette, transforming a host cell with the cassette, and allowing homologous recombination between the promoter cassette and homologous flanking regions of the target site, where the cassette replaces a native promoter region of a chromosomal gene of interest as compared to the expression of the chromosomal gene of interest in a corresponding parent host cell; and (7) an isolated promoter comprising a fully defined sequence of 49 or 51 base pairs, as given in the specification.

BIOTECHNOLOGY - Preferred Method: Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest further comprises selecting transformed bacterial cells from the library. The host cells are selected from Escherichia coli, **Bacillus** sp. and Pantoea sp. The selected bacterial cells have a higher or lower level of expression of the gene of interest than bacterial cells comprising the precursor promoter. The promoter library comprises the Ptrc, Ptac or PGI precursor promoter and modified Ptrc, Ptac or PGI precursor promoters. The promoter library comprises modified promoters having a sequence of 49 base pairs fully defined in the specification. Modifying the regulatory function of a native promoter of a chromosomal gene of interest further comprises excising the selectable marker from the transformed host cell, and isolating the transformed host cell. Preferred Promoter Cassette: The precursor promoter is selected from Ptrc, Ptacl, PD/E20, PH207, PN25, PG25, PJ5, PA1, PA2, PA3, PL, Plac, PlacUV5, Pcon and Pbla. The -35 region of the precursor promoter is selected from **TTGACA**, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA

and TTCAAA. The -10 region of the precursor promoter is selected from TAAGAT, **TATAAT**, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT. The -35 region of the precursor promoter is **TTGACA** and the -10 region of the precursor promoter is **TATAAT** or AATAAT. The linker sequence of the precursor promoter is modified. The first and second recombinase recognition sites are non-identical recombinase sites and selected from lox and mutant lox sites. The modified precursor promoter is selected from NF-T, NF-G, NF-C, NF-1T and NF-2T. The NF-T, NF-G and NF-C each comprise a fully defined sequence comprising 49 base pairs, as given in the specification. The NF-1T and NF-2T each comprise a fully defined sequence of 51 base pairs, as given in the specification.

USE - The method is useful in constructing a library of promoters to be introduced into bacterial host cells, which results in a population of transformed bacterial cells having a range of gene expression (claimed). The method is used in developing a quick and efficient means of determining the optimum expression level of a gene in a metabolic pathway which, in turn, results in an optimization of strain performance for a desired product. (52 pages)

L13 ANSWER 5 OF 15 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in

which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L13 ANSWER 6 OF 15 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter

and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L13 ANSWER 7 OF 15 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a **Bacillus** cell.
AUTHOR(S): Widner, William [Inventor, Reprint author]; Sloma, Alan [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L13 ANSWER 8 OF 15 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001057967 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10896218
TITLE: Analysis of promoter sequences from Lactobacillus and Lactococcus and their activity in several Lactobacillus species.
AUTHOR: McCracken A; Turner M S; Giffard P; Hafner L M; Timms P
CORPORATE SOURCE: Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Australia.
SOURCE: Archives of microbiology, (2000 May-Jun) 173 (5-6) 383-9. Journal code: 0410427. ISSN: 0302-8933.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200012
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001221

AB Promoter-active fragments were isolated from the genome of the probiotic

organism Lactobacillus rhamnosus strain GG using the promoter-probe vector pNZ272. These promoter elements, together with a promoter fragment isolated from the vaginal strain Lactobacillus fermentum BR11 and two previously defined promoters (Lactococcus lactis and Lactobacillus acidophilus ATCC 4356 slpA), were introduced into three strains of Lactobacillus. Primer-extension analysis was used to map the transcriptional start site for each promoter. All promoter fragments tested were functional in each of the three lactobacilli and a purine residue was used to initiate transcription in most cases. The promoter elements encompassed a 52- to 1,140-fold range in promoter activity depending on the host strain. Lactobacillus promoters were further examined by surveying previously mapped sequences for conserved base positions. The Lactobacillus hexamer regions (-35: **TTgaca** and -10: **TAtAAT**) closely resembled those of Escherichia coli and **Bacillus** subtilis, with the highest degree of agreement at the -10 hexamer. The TG dinucleotide upstream of the -10 hexamer was conserved in 26% of Lactobacillus promoters studied, but conservation rates differed between species. The region upstream of the -35 hexamer of Lactobacillus promoters showed conservation with the bacterial UP element.

L13 ANSWER 9 OF 15 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4

ACCESSION NUMBER: 2000:254695 SCISEARCH
THE GENUINE ARTICLE: 297YY
TITLE: Inferring regulatory elements from a whole genome. An analysis of Helicobacter pylori sigma(80) family of promoter signals
AUTHOR: Vanet A; Marsan L; Labigne A; Sagot M F (Reprint)
CORPORATE SOURCE: INST PASTEUR, SERV INFORMAT SCI, 28 RUE DR ROUX, F-75724 PARIS, FRANCE (Reprint); INST PASTEUR, SERV INFORMAT SCI, F-75724 PARIS, FRANCE; INST BIOL PHYSICOCHIM, CNRS, UPR 9073, F-75005 PARIS, FRANCE; INST PASTEUR, UNITE PATHOGENIE BACTERIENNE MUSQUEUSES, F-75724 PARIS 15, FRANCE; INST GASPARD MONGE, MARNE VALLEE, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (24 MAR 2000) Vol. 297, No. 2, pp. 335-353.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0022-2836.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 45

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Helicobacter pylori is adapted to life in a unique niche, the gastric epithelium of primates. Its promoters may therefore be different from those of other bacteria. Here, we determine motifs possibly involved in the recognition of such promoter sequences by the RNA polymerase using a new motif identification method. An important feature of this method is that the motifs are sought with the least possible assumptions about what they may look like. The method starts by considering the whole genome of H. pylori and attempts to infer directly from it a description for a family of promoters. Thus, this approach differs from searching for such promoters with a previously established description. The two algorithms are based on the idea of inferring motifs by flexibly comparing words in the sequences with an external object, instead of between themselves. The first algorithm infers single motifs, the second a combination of two motifs separated from one another by strictly defined, sterically constrained distances. Besides independently finding motifs known to be present in other bacteria, such as the Shine-Dalgarno sequence and the TATA-box, this approach suggests the existence in H. pylori of a new, combined motif, TTAAGC, followed optimally 21 bp downstream by

TATAAT. Between these two motifs, there is in some cases another, TTTTAA or, less frequently, a repetition of TTAAGC separated optimally from the TATA-box by 12 bp. The combined motif TTAAGC x (21 +/- 2) **TATAAT** is present with no errors immediately upstream from the only two copies of the ribosomal 23 S-5 S RNA genes in H. pylori, and with one error upstream from the only two copies of the ribosomal 16 S RNA genes. The operons of both ribosomal RNA molecules are strongly expressed, representing an encouraging sign of the pertinence of the motifs found by the algorithms. In 25 cases out of a possible 30, the combined motif is found with no more than three substitutions immediately upstream from ribosomal proteins, or operons containing a ribosomal protein. This is roughly the same frequency of occurrence as for **TTGACA** x (15-19) **TATAAT** (with the same maximum number of substitutions allowed) described as being the sigma(70) promoter sequence consensus in **Bacillus** subtilis and Escherichia coli. The frequency of occurrence of the new motif obtained, TTAAGC x (19-23)**TATAAT**, remains high when all protein genes in H. pylori are considered, as is the case for the **TTGACA** x (15-19)**TATAAT** motif in B. subtilis but not in E. coli. (C) 2000 Academic Press.

L13 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412
PRIORITY APPLN. INFO.:			US 1998-31442 A	19980226
			US 1999-256377 B3	19990224
			WO 1999-US4360 W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from

the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

L13 ANSWER 11 OF 15 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:776497 SCISEARCH
THE GENUINE ARTICLE: 243PM
TITLE: Efficiency of transcription from promoter sequence variants in Lactobacillus is both strain and context dependent
AUTHOR: McCracken A; Timms P (Reprint)
CORPORATE SOURCE: QUEENSLAND UNIV TECHNOL, SCH LIFE SCI, CTR MOL BIOTECHNOL, GPO BOX 2434, BRISBANE, QLD 4001, AUSTRALIA (Reprint); QUEENSLAND UNIV TECHNOL, SCH LIFE SCI, CTR MOL BIOTECHNOL, BRISBANE, QLD 4001, AUSTRALIA
COUNTRY OF AUTHOR: AUSTRALIA
SOURCE: JOURNAL OF BACTERIOLOGY, (OCT 1999) Vol. 181, No. 20, pp. 6569-6572.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 21

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The introduction of consensus -35 (**TTGACA**) and -10 (**TATAAT**) hexamers and a TG motif into the Lactobacillus acidophilus ATCC 4356 wild-type slpA promoter resulted in significant improvements (4.3-, 4.1-, and 10.7-fold, respectively) in transcriptional activity in Lactobacillus fermentum BR11. In contrast, the same changes resulted in decreased transcription in Lactobacillus rhamnosus GG. The TG motif was shown to be important in the context of weak -35 and -10 hexamers (L. fermentum BR11) or a consensus -10 hexamer (L. rhamnosus GG). Thus, both strain- and context-dependent effects are critical factors influencing transcription in Lactobacillus.

L13 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 95047234 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7958762
TITLE: Determination and comparison of Lactobacillus delbrueckii ssp. lactis DSM7290 promoter sequences.
AUTHOR: Matern H T; Klein J R; Henrich B; Plapp R
CORPORATE SOURCE: Universitat Kaiserslautern, Fachbereich Biologie, Abteilung Mikrobiologie, FRG.
SOURCE: FEMS microbiology letters, (1994 Sep 15) 122 (1-2) 121-8.

Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 19950110
Entered Medline: 19941128

AB The transcriptional start points of ten Lactobacillus delbruckii ssp. lactis DSM7290 genes were determined by primer extension. The upstream located promoter regions, including potential -35 and -10 regions and the spacing between them were compared to the well-known Escherichia coli and **Bacillus** subtilis promoters. The Lb. delbruckii -35 consensus sequence (**TTGACA**) seems to be less conserved then the E. coli sequence. The nucleotides TGC were often found upstream of the -10 region (**TATAAT**). The most frequently observed spacing between the two core promoter regions was 17 nt and the main distance between the -10 region and the transcriptional start point was mostly determined to be 6 nt in contrast to 7 nt, as described for E. coli promoters. The preferred initiation nucleotides in Lb. delbruckii were shown to be definitely purines (A or G). The ribosome binding sites located downstream of the promoters revealed the consensus sequence 3'-UCCUCCU-5', being the predicted 3'-OH end of the Lactobacillus 16S rRNA with a high degree of homology to known 16S rRNAs.

L13 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 90362027 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2391488
TITLE: Nucleotide sequence of the alpha-amylase-pullulanase gene from Clostridium thermohydrosulfuricum.
AUTHOR: Melasniemi H; Paloheimo M; Hemio L
CORPORATE SOURCE: Research Laboratories, Alko Ltd., Helsinki, Finland.
SOURCE: Journal of general microbiology, (1990 Mar) 136 (3) 447-54.
Journal code: 0375371. ISSN: 0022-1287.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M28471
ENTRY MONTH: 199010
ENTRY DATE: Entered STN: 19901109
Last Updated on STN: 19901109
Entered Medline: 19901001

AB The nucleotide sequence of the gene (apu) encoding the thermostable alpha-amylase-pullulanase of Clostridium thermohydrosulfuricum was determined. An open reading frame of 4425 bp was present. The deduced polypeptide (Mr 165,600), including a 31 amino acid putative signal sequence, comprised 1475 amino acids, with no cysteine residues. The structural gene was preceded by the consensus promoter sequence **TTGACA TATAAT**, a putative regulatory sequence and a putative ribosome-binding sequence AAAGGGGG. The codon usage resembled that of **Bacillus** genes. The deduced sequence of the mature apu product showed similarities to various amylolytic enzymes, especially the neopullulanase of **Bacillus** stearothermophilus, whereas the signal sequence showed similarity to those of the alpha-amylases of B. stearothermophilus and B. subtilis. Three regions thought to be highly conserved in the primary structure of alpha-amylases could also be distinguished in the apu product, two being partly 'duplicated' in this alpha-1,4/alpha-1,6-active enzyme.

L13 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 86135998 MEDLINE

DOCUMENT NUMBER: PubMed ID: 3937729
TITLE: In vivo transfer of genetic information between gram-positive and gram-negative bacteria.
AUTHOR: Trieu-Cuot P; Gerbaud G; Lambert T; Courvalin P
SOURCE: EMBO journal, (1985 Dec 16) 4 (13A) 3583-7. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198604
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860414

AB A 1427-bp DNA fragment containing the kanamycin resistance gene, aphA-3, of plasmid pIP1433 from Campylobacter coli was inserted into a shuttle vector. Full expression of aphA-3 was obtained in **Bacillus** subtilis and in Escherichia coli. This DNA fragment was sequenced in its entirety and the starting point for aphA-3 transcription in B. subtilis, C. coli and E. coli was determined by S1 nuclease mapping. The sequence of the promoter consists of the hexanucleotides **TTGACA** and **TATAAT**, with a spacing of 17 bp. The nucleotide sequence of the aphA-3 gene from C. coli and from the streptococcal plasmid pJH1 are identical whereas they differ by two substitutions and deletion of a codon from that cloned from the staphylococcal plasmid pSH2. These results indicate a recent extension of the resistant gene pool of Gram-positive cocci to Gram-negative **bacilli**. From an analysis of the DNA sequences surrounding the promoter region, we concluded that the DNA fragment containing the aphA-3 gene in plasmid pJH1 has evolved by deletions from a sequence similar to that found in plasmid pIP1433.

L13 ANSWER 15 OF 15 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 83012205 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6181373
TITLE: Nucleotide sequences that signal the initiation of transcription and translation in **Bacillus** subtilis.
AUTHOR: Moran C P Jr; Lang N; LeGrice S F; Lee G; Stephens M; Sonenshein A L; Pero J; Losick R
CONTRACT NUMBER: GM18568 (NIGMS)
GM19168 (NIGMS)
SOURCE: Molecular & general genetics : MGG, (1982) 186 (3) 339-46. Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J01550; GENBANK-J01552; GENBANK-J01553
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19821202

AB We have determined the nucleotide sequence of two **Bacillus** subtilis promoters (veg and tms) that are utilized by the principal form of B. subtilis RNA polymerase found in vegetative cells (sigma 55-RNA polymerase) and have compared our sequences to those of several previously reported **Bacillus** promoters. Hexanucleotide sequences centered approximately 35 (the "--35" region) and 10 (the "--10" region) base pairs upstream from the veg and tms transcription starting points (and separated by 17 base pairs) corresponded closely to the consensus hexanucleotides (**TTGACA** and **TATAAT**) attributed to Escherichia coli promoters. Conformity to the preferred --35 and --10 sequences may not be sufficient to promote efficient utilization by B. subtilis RNA polymerase,

however, since three promoters (veg, tms and E. coli tac) that conform to these sequences and that are utilized efficiently by E. coli RNA polymerase were used with highly varied efficiencies by B. subtilis RNA polymerase. We have also analyzed mRNA sequences in DNA located downstream from eight B. subtilis chromosomal and phage promoters for nucleotide sequences that might signal the initiation of translation. In accordance with the rules of McLaughlin, Murray and Rabinowitz (1981), we observe mRNA nucleotide sequences with extensive complementarity to the 3' terminal region of B. subtilis 16S rRNA, followed by an initiation codon and an open reading frame.

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=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1        377498 S BACILLUS
L2             0 S ALPHA(A)AMYOLASE
L3         52121 S ALPHA(A)AMYLASE
L4         10118 S L1 AND L3
L5            77 S "AMYQ"
L6            57 S L4 AND L5
L7            23 DUP REM L6 (34 DUPLICATES REMOVED)
L8           310 S "TTGACA"
L9             1 S L7 AND L8
L10          428 S "TATAAT"
L11          109 S L8 AND L10
L12           35 S L1 AND L11
L13           15 DUP REM L12 (20 DUPLICATES REMOVED)

=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1        377498 S BACILLUS
L2             0 S ALPHA(A)AMYOLASE
L3         52121 S ALPHA(A)AMYLASE
L4         10118 S L1 AND L3
L5            77 S "AMYQ"
L6            57 S L4 AND L5
L7            23 DUP REM L6 (34 DUPLICATES REMOVED)
L8           310 S "TTGACA"
L9             1 S L7 AND L8
L10          428 S "TATAAT"
L11          109 S L8 AND L10
L12           35 S L1 AND L11
L13           15 DUP REM L12 (20 DUPLICATES REMOVED)

=> s l5 and promoter
L14           26 L5 AND PROMOTER

=> ddup rem l14
DDUP IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> dup rem l14
PROCESSING COMPLETED FOR L14
```

L15 13 DUP REM L14 (13 DUPLICATES REMOVED)

=> d 1-13 ibib ab

L15 ANSWER 1 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one **promoter** that is a consensus **promoter** having the sequence TTGACA for the -35 region and TATAAT for the -10 region, and/or which is derived from amyL, **amyQ**, amyM, cryIIIA, dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The **promoter** is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and

3' end of the 5' and 3' flanking segments, respectively. The **promoter** is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is Bacillus subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the Bacillus subtilis genome or in the cryIIIa **promoter**. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a Bacillus host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L15 ANSWER 2 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus **promoter** is obtained from a **promoter** obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (**amyQ**). The mRNA processing/stabilizing sequence is the cryIIIA

mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem **promoter**, in which each **promoter** sequence of the tandem **promoter** is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem **promoter** and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus **promoter** having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus **promoter**, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus **promoter** is obtained from any bacterial or a bacillus **promoter**. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L15 ANSWER 3 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-22251 BIOTECHDS
TITLE: Modulating Sec-dependent protein secretion, comprises introducing a spoIIIJ or yqjG gene linked to an inducible **promoter** into a Bacillus cell and modulating the expression of the spoIIIJ or yqjG gene;
vector-mediated gene transfer and expression in host cell for strain improvement
AUTHOR: BRON S; TJALSMA H; VAN DIJL J M
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003060068 24 Jul 2003
APPLICATION INFO: WO 2002-US39634 12 Dec 2002
PRIORITY INFO: US 2002-426832 15 Nov 2002; US 2002-348080 9 Jan 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-598514 [56]
AB DERWENT ABSTRACT:

NOVELTY - Modulating Sec-dependent protein secretion comprising introducing a spoIIIJ or yqjG gene linked to an inducible **promoter** into a Bacillus cell, and modulating the expression of the spoIIIJ or yqjG gene by varying the level of induction of the inducible **promoter**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a purified DNA molecule comprising an inducible **promoter** operatively linked to the spoIIIJ or yqjG gene; and (2) a method of

modulating the secretion of a protein of interest comprising forming a first DNA molecule encoding a chimeric protein comprising a Sec-dependent secretion signal peptide, forming a second DNA molecule encoding an inducible **promoter** operably linked to the spoIIIJ or yqjG gene, transforming a host cell with the DNA molecule, and growing the host cell under conditions where the protein of interest is expressed at the desired level.

WIDER DISCLOSURE - Methods of inhibiting sporulation in a Bacillus cell comprising a mutation of the spoIIIJ gene, where the mutation results in the formation of an inactive gene product, are also disclosed.

BIOTECHNOLOGY - Preferred Method: Alternatively, modulating Sec-dependent protein secretion comprises providing a Bacillus cell comprising spoIIIJ and yqjG genes linked to an endogenous high expression **promoter**, and modulating the expression of the spoIIIJ and yqjG genes by varying the level of induction of the **promoter**. The (inducible) **promoter** is the Pspac **promoter**. In modulating the secretion of a protein of interest, the host cell is grown under conditions where the inducible **promoter** is induced. The protein of interest is expressed at low level.

USE - The methods are useful for enhancing the secretion of proteins from a host cell, preferably from a Bacillus cell, that may be made to be secreted via the Sec-dependent secretion pathway. The DNA molecules are useful for the inducible expression of the spoIIIJ and/or yqjG genes.

EXAMPLE - To evaluate the importance of yqjG and spoIIIJ function for protein secretion, Bacillus subtilis DELTAyqjG, DELTAspoIIIJ and DELTAyqjG-IspoIIIJ, as well as the parental strain 168 were transformed with plasmid pLip2031 for the secretion of the B. subtilis lipase LipA, pPSPPhoA5 for the secretion of the alkaline phosphatase PhoA of Escherichia coli fused to the prepro-region of the lipase gene from Staphylococcus hyicus, or pKTH10 for the secretion of the alpha-amylase **AmyQ**. In order to deplete B. subtilis DELTAyqjG-IspoIIIJ of spoIIIJ, this strain was grown for 3 hours in tryptone/yeast extract (TY) medium without isopropyl-beta-D-thiogalacto-pyranoside (IPTG). As a control, TY medium with 50 nM IPTG or 500 nM IPTG was used. The secretion of LipA, PhoA and **AmyQ** was analyzed by Western blotting. The levels of LipA, PhoA and **AmyQ** in the medium of spoIIIJ-depleted cells of B. subtilis DELTAyqjG-IspoIIIJ (no IPTG) were significantly reduce compared to those in the media of the fully induced double mutant (500 nM IPTG), or the parental strain 168. The levels of the LipA and PhoA in the media of DELTAyqjG-IspoIIIJ strains that were fully induced with IPTG (500 nM) were higher than those in the media of the parental control strains. This suggests that over expression of the spoIIIJ gene can result in improved protein secretion in B. subtilis.(50 pages)

L15 ANSWER 4 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem **promoter**;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:
NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the

Bacillus cell comprises a nucleic acid construct comprising a tandem **promoter** in which each **promoter** sequence of the tandem **promoter** is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem **promoter** in which each **promoter** sequence of the tandem **promoter** is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem **promoter** and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem **promoter** and upstream of the nucleic acid sequence encoding the polypeptide. The tandem **promoter** comprises two or more bacterial **promoter** sequences, which are obtained from one or more Bacillus genes. The tandem **promoter** comprises the **amyQ promoter**, a consensus **promoter** having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL **promoter**, and/or the cryIIIA **promoter**. The tandem **promoter** comprises two copies of the **amyQ**, amyl or cryIIIA **promoter**. The two or more **promoter** sequences of the tandem **promoter** simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more **promoter** sequences of the tandem **promoter** promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus **promoter** having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus **promoter** and upstream of the nucleic acid sequence encoding the polypeptide; and

isolating the polypeptide from the cultivation medium. The consensus **promoter** is obtained from any bacterial **promoter**, preferably a Bacillus **promoter**. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L15 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:511475 HCAPLUS
DOCUMENT NUMBER: 139:80184
TITLE: Recombinant expression of bacterial hyaluronan synthase operon genes in Bacillus and hyaluronic acid production
INVENTOR(S): Sloma, Alan; Behr, Regine; Widner, William; Tang, Maria; Sternberg, David; Brown, Stephen
PATENT ASSIGNEE(S): Novozymes Biotech, Inc., USA
SOURCE: PCT Int. Appl., 218 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003054163	A2	20030703	WO 2002-US41067	20021220
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003175902	A1	20030918	US 2002-326185	20021220
PRIORITY APPLN. INFO.:			US 2001-342644P	P 20011221

AB The present invention relates to methods for producing a hyaluronic acid, comprising: (a) cultivating a Bacillus host cell under conditions suitable for production of the hyaluronic acid, wherein the Bacillus host cell comprises a nucleic acid construct comprising a hyaluronan synthase encoding sequence operably linked to a **promoter** sequence foreign to the hyaluronan synthase encoding sequence; and (b) recovering the hyaluronic acid from the cultivation medium. The present invention also relates to an isolated nucleic acid sequence encoding a hyaluronan synthase operon comprising a hyaluronan synthase gene and a UDP-glucose 6-dehydrogenase gene, and optionally one or more genes selected from the group consisting of a UDP-glucose pyrophosphorylase gene, UDP-N-acetylglucosamine pyrophosphorylase gene, and glucose-6-phosphate isomerase gene. The present invention also relates to isolated nucleic acid sequences of genes encoding a UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, and UDP-N-acetylglucosamine pyrophosphorylase. Hyaluronic acid obtained by the methods of this invention and GPC (gel permeation or size-exclusion chromatog.) has a mol. weight of about 1-4 megaDaltons.

L15 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:457872 HCAPLUS

DOCUMENT NUMBER: 139:163626
TITLE: Production of Chlamydia pneumoniae proteins in Bacillus subtilis and their use in characterizing immune responses in the experimental infection model
AUTHOR(S): Airaksinen, Ulla; Penttila, Tuula; Wahlstrom, Eva; Vuola, Jenni M.; Puolakkainen, Mirja; Sarvas, Matti
CORPORATE SOURCE: Department of Vaccines, National Public Health Institute, Helsinki, Finland
SOURCE: Clinical and Diagnostic Laboratory Immunology (2003), 10(3), 367-375
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Due to intracellular growth requirements, large-scale cultures of chlamydiae and purification of its proteins are difficult and laborious. To overcome these problems we produced chlamydial proteins in a heterologous host, Bacillus subtilis, a gram-pos. nonpathogenic bacterium. The genes of Chlamydia pneumoniae major outer membrane protein (MOMP), the cysteine-rich outer membrane protein (Omp2), and the heat shock protein (Hsp60) were amplified by PCR, and the PCR products were cloned into expression vectors containing a **promoter**, a ribosome binding site, and a truncated signal sequence of the α-amylase gene from Bacillus amyloliquefaciens. C. pneumoniae genes were readily expressed in B. subtilis under the control of the α-amylase **promoter**. The recombinant proteins MOMP and Hsp60 were purified from the bacterial lysate with the aid of the carboxy-terminal histidine hexamer tag by affinity chromatog. The Omp2 was separated as an insol. fraction after 8 M urea treatment. The purified proteins were successfully used as immunogens and as antigens in serol. assays and in a lymphoproliferation test. The Omp2 and Hsp60 antigens were readily recognized by the antibodies appearing after pulmonary infection following intranasal inoculation of C. pneumoniae in mice. Also, splenocytes collected from mice immunized with MOMP or Hsp60 proteins proliferated in response to in vitro stimulation with the corresponding proteins.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:91455 HCAPLUS
DOCUMENT NUMBER: 134:143860
TITLE: A pectin acetylesterase of Bacillus subtilis and cloning and expression of the yxiM gene encoding it
INVENTOR(S): Thomas, Michael D.; Brown, Kimberly M.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: U.S., 35 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6184028	B1	20010206	US 1999-384305	19990826
WO 2001014534	A2	20010301	WO 2000-US23521	20000825

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,

DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1212435 A2 20020612 EP 2000-968327 20000825

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.: US 1999-384305 A 19990826

WO 2000-US23521 W 20000825

AB The present invention relates to isolated polypeptides having pectin acetylesterase activity and isolated nucleic acid sequences encoding the polypeptides. The gene is an allele of the yxiM gene. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides. The gene was cloned by PCR using primers derived from known yxiM sequence and the primers contained sequences that allowed to be cloned directly under control of the **promoter** of the subtilisin gene. Construction of a number of expression vectors for high level expression of the gene in B. subtilis is described. Fermentation of the catalytically active protein is demonstrated.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 8 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2001-01575 BIOTECHDS

TITLE: Immunity to Chlamydia pneumoniae induced by vaccination with DNA vector expressing a cytoplasmic protein (Hsp60) or outer membrane proteins (MOMP and Omp2);
nucleic acid vaccine, cysteine cytoplasmic protein and outer membrane proteins useful for inducing immune response

AUTHOR: Penttila T; Vuola J M; Puurula V; Anttila M; Anttila M; Sarvas M; Rautonen N; Makela P H; Puolakkainen M

CORPORATE SOURCE: Univ.Helsinki; Nat.Public-Health-Inst.Helsinki; Nat.Vet.Food-Res.Inst.Helsinki

LOCATION: Department of Virology, POB 21, Haartman Institute ,University of Helsinki, FIN-00014 Helsinki Finland. Email: tuula.penttila@helsinki.fi

SOURCE: Vaccine; (2000) 19, 9-10, 1256-65
CODEN: VACCDE
ISSN: 0264-410X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Immunity to Chlamydia pneumoniae induced by vaccination with DNA vectors expressing a cytoplasmic protein (Hsp60) or outer membrane protein (MOMP and Omp2), was studied. Mycoplasma-free C. pneumoniae K6 was propagated in HL cell in minimal essential medium with 10% fetal cattle serum and 0.3 mg/ml L-glutamine. Recombinant C. pneumoniae protein MOMP, Omp2 and Hsp60 were produced in Bacillus subtilis. The momp, omp2 and Hsp60 were amplified by polymerase chain reaction, and cloned into the expression vector containing the **promoter**, RBS and a short 5' stretch of the alpha-amylase (EC-3.2.1.1) gene (**amyQ**). C. pneumoniae genes encoding for MOMP, Omp2 and Hsp60 were cloned into an eukaryotic expression vector plasmid pcDNA3.1. Immunization with pmomp or phsp60 showed 1.2-1.5 log reduction in the mean lung bacterial counts after the challenge. Specific antibodies were detected only in sera of mice immunized with pmomp2 and phsp60. Immunization with any of the three vaccines did not reduce the severity of histologically assessed pneumonia, but resulted in significantly higher lymphoid reaction in the lung indicating immunological memory. (43 ref)

L15 ANSWER 9 OF 13 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3

ACCESSION NUMBER: 2001030732 EMBASE

TITLE: Development of marker-free strains of Bacillus subtilis

capable of secreting high levels of industrial enzymes.
AUTHOR: Widner B.; Thomas M.; Sternberg D.; Lammon D.; Behr R.; Sloma A.
CORPORATE SOURCE: Dr. B. Widner, Novo Nordisk Biotech., Inc., Davis, CA 95616, United States. wwidner@nnbt.com
SOURCE: Journal of Industrial Microbiology and Biotechnology, (2000) Vol. 25, No. 4, pp. 204-212.
Refs: 31
ISSN: 1367-5435 CODEN: JIMBFL
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20010208
Last Updated on STN: 20010208

AB Different strategies have been employed to achieve high-level expression of single-copy genes encoding secreted enzymes in Bacillus subtilis. A model system was developed which utilizes the aprL gene from Bacillus clausii as a reporter gene for monitoring expression levels during stationary phase. An exceptionally strong **promoter** was constructed by altering the nuceotide sequence in the -10 and -35 regions of the **promoter** for the **amyQ** gene of Bacillus amyloliquefaciens. In addition, two or three tandem copies of this **promoter** were shown to increase expression levels substantially in comparison to the monomer **promoter** alone. Finally, the **promoter** and mRNA stabilization sequences derived from the cry3A gene of Bacillus thuringiensis were used in combination with the mutant **amyQ promoter** to achieve the highest levels of aprL expression. These **promoters** were shown to be fully functional in a high-expressing Bacillus strain grown under industrial fermentation conditions. The ability to obtain maximum expression levels from a single copy gene now makes it feasible to construct environmentally friendly, marker-free industrial strains of B. subtilis.

L15 ANSWER 10 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 4
ACCESSION NUMBER: 1999-15556 BIOTECHDS
TITLE: Production of polypeptide in Bacillus sp. using specific **promoters**, particularly for producing enzymes; the effect of a short consensus **amyQ promoter** on recombinant alpha-amylase production via vector-mediated gene transfer and expression in Bacillus subtilis
AUTHOR: Widner W; Sloma A; Thomas M D
PATENT ASSIGNEE: Novo-Nordisk-Biotech
LOCATION: Davis, CA, USA.
PATENT INFO: WO 9943835 2 Sep 1999
APPLICATION INFO: WO 1999-US4360 26 Feb 1999
PRIORITY INFO: US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-561370 [47]

AB The production of a protein (I) in Bacillus sp. cells using specific tandem or consensus **promoters** is new. Also claimed are: the production of the recombinant Bacillus sp. cells via the introduction of a nucleic acid construct; the production of Bacillus sp. mutants which contain no selectable marker genes by treating the cells to delete a marker gene; marker-free mutant cell produced using this method; isolated consensus alpha-amylase (**amyQ**) **promoter** sequence made up of 2 185 bp DNA sequences (specified); a nucleic acid construct containing a sequence (II), which encodes (I), linked to one or more copies of the **amyQ promoter**; and a recombinant vector

and Bacillus sp. cells containing this construct. This new method may be useful for producing homologs or particularly heterologous proteins, particularly enzymes (specifically serine protease, maltogenic alpha-amylase, EC-3.2.1.1 and pullulanase, EC-3.2.1.41), but also hormones, antibodies, reporters, etc. In an example, the replacement of the **amyQ promoter** with a short consensus **amyQ promoter** resulted in a increase in enzyme expression of 620% in Bacillus subtilis strain PL801 cells. (89pp)

L15 ANSWER 11 OF 13 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1999157560 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10027970
TITLE: Ecs, an ABC transporter of Bacillus subtilis: dual signal transduction functions affecting expression of secreted proteins as well as their secretion.
AUTHOR: Leskela S; Wahlstrom E; Hyyrylainen H L; Jacobs M; Palva A; Sarvas M; Kontinen V P
CORPORATE SOURCE: Vaccine Development Laboratory, National Public Health Institute, Helsinki, Finland.
SOURCE: Molecular microbiology, (1999 Jan) 31 (2) 533-43. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429

AB ecs is a three-cistron operon of Bacillus subtilis, encoding proteins with similarity to the ATPase (EcsA) and hydrophobic components (EcsB) of ABC transporters. The ecsA26 point mutation was shown to cause a strong processing defect of a secreted alpha-amylase precursor (preAmyQ) and of three other exoproteins. Northern analysis of the level of **amyQ** mRNA showed that ecsA26 also decreases **amyQ** transcription. This effect too was pleiotropic, as judged by a drastic decrease in the expression from an exoprotease **promoter** of a reporter protein. A knockout mutation of the ecsB cistron caused a processing defect similar to ecsA26 but, unlike ecsA26, did not affect **amyQ** transcription. These was also no defect in transcription in the ecsA ecsB double mutant. Thus, an intact ecsB product was required for the downregulation of **amyQ** by the mutant ecsA. These results suggest a dual regulatory function for Ecs, in which Ecs, possibly as part of a signal transduction mechanism, regulates some component(s) of the protein secretion apparatus as well as secretory protein transcription in a co-ordinated fashion.

L15 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:352949 HCAPLUS
DOCUMENT NUMBER: 129:27099
TITLE: Methods for producing polypeptides in surfactin mutants of Bacillus cells
INVENTOR(S): Sloma, Alan; Sternberg, David; Adams, Lee F.; Brown, Stephen
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9822598 A1 19980528 WO 1997-US21084 19971118
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9854450 A1 19980610 AU 1998-54450 19971118
EP 941349 A1 19990915 EP 1997-948365 19971118
EP 941349 B1 20030730
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI
CN 1240482 A 20000105 CN 1997-180644 19971118
JP 2001503641 T2 20010321 JP 1998-523825 19971118
AT 246251 E 20030815 AT 1997-948365 19971118
PRIORITY APPLN. INFO.: US 1996-749521 A 19961118
US 1997-49441P P 19970612
US 1996-749421 A 19961118
WO 1997-US21084 W 19971118

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a mutant of a Bacillus cell, wherein the mutant (i) comprises a first nucleic acid sequence encoding the polypeptide and a second nucleic acid sequence comprising a modification of at least one of the genes responsible for the biosynthesis or secretion of a surfactin or isoform thereof under conditions conducive for the production of the polypeptide and (ii) the mutant produces less of the surfactin or isoform thereof than the Bacillus cell when cultured under the same conditions; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to mutants of Bacillus cells and methods for producing the mutants. B. subtilis ΔspoIIAc ΔnprE ΔaprE ΔamyE ΔsrfC strains were prepared and transformed with an **amyQ promoter**-amyM chimeric gene. Culture of these strains resulted in less foaming and resultant volume loss than culture of strains containing the srfC gene.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 13 OF 13 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-01709 BIOTECHDS
TITLE: Cloning and expression of an amylase gene from Bacillus stearothermophilus;
thermostable alpha-amylase expression in Bacillus subtilis and Bacillus licheniformis (conference paper)
AUTHOR: Diderichsen B; Poulsen G B; Jorgensen P L
CORPORATE SOURCE: Novo-Nordisk
LOCATION: Novo Nordisk A/S, DK-2880 Bagsvaerd, Denmark.
SOURCE: Res.Microbiol.; (1991) 142, 7-8, 793-96
CODEN: RMCREW
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Bacillus stearothermophilus alpha-amylase (EC-3.2.1.1) gene, amyS, was cloned and expressed in Bacillus subtilis under its own expression signals. The AmyS yield was 200-fold higher than in the B. stearothermophilus donor. However, compared to other alpha-amylases cloned in B. subtilis, yields were low. Yields were increased 4-fold by the insertion of 2 **promoters** (Pm and Pq from the amyM gene of B. stearothermophilus and Bacillus amyloliquefaciens, respectively) in tandem, upstream of the amyS **promoter**. A suitable plasmid harboring amyS transcribed by the amyM and **amyQ promoters** was introduced by protoplast transformation into a Bacillus licheniformis strain that expressed negligible amounts of AmyL. The resulting strain showed a 3-fold increase in AmyS productivity compared to an equivalent B. subtilis construction. Replacement of the amyS **promoter**, ribosome binding site and signal peptide with

the corresponding functions from amyL did not increase yields further. Thermostable alpha-amylase is used for the industrial production of glucose or high fructose syrups. (12 ref)

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=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1       377498 S BACILLUS
L2            0 S ALPHA(A)AMYOLASE
L3        52121 S ALPHA(A)AMYLASE
L4        10118 S L1 AND L3
L5           77 S "AMYQ"
L6           57 S L4 AND L5
L7           23 DUP REM L6 (34 DUPLICATES REMOVED)
L8          310 S "TTGACA"
L9            1 S L7 AND L8
L10         428 S "TATAAT"
L11         109 S L8 AND L10
L12          35 S L1 AND L11
L13          15 DUP REM L12 (20 DUPLICATES REMOVED)
L14          26 S L5 AND PROMOTER
L15          13 DUP REM L14 (13 DUPLICATES REMOVED)

=> e widner w/au
E1           32     WIDNER T E/AU
E2            2     WIDNER THOMAS E/AU
E3           30 --> WIDNER W/AU
E4            7     WIDNER W E/AU
E5           43     WIDNER W R/AU
E6           13     WIDNER WILLIAM/AU
E7           18     WIDNER WILLIAM R/AU
E8            1     WIDNER WILLIAM ROY/AU
E9            1     WIDNER WM R/AU
E10           5     WIDNES C/AU
E11           2     WIDNES J/AU
E12           2     WIDNES J A/AU

=> s e3-e9
L16         113 ("WIDNER W"/AU OR "WIDNER W E"/AU OR "WIDNER W R"/AU OR "WIDNER
                WILLIAM"/AU OR "WIDNER WILLIAM R"/AU OR "WIDNER WILLIAM ROY"/AU
                OR "WIDNER WM R"/AU)

=> e sloma a/au
E1            3     SLOM T J/AU
E2            2     SLOM TREVOR J/AU
E3          120 --> SLOMA A/AU
E4           15     SLOMA A P/AU
E5           55     SLOMA ALAN/AU
E6           12     SLOMA ALAN P/AU
E7            1     SLOMA ALAN PAUL/AU
E8            1     SLOMA D/AU
E9            1     SLOMA D R/AU
E10           6     SLOMA E/AU
E11           2     SLOMA E J/AU
E12           3     SLOMA I/AU

=> s e3-e7
L17         203 ("SLOMA A"/AU OR "SLOMA A P"/AU OR "SLOMA ALAN"/AU OR "SLOMA
                ALAN P"/AU OR "SLOMA ALAN PAUL"/AU)
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=> e thomas m d/au
E1            4      THOMAS M C C/AU
E2           12      THOMAS M CARMEN/AU
E3          395 --> THOMAS M D/AU
E4          100      THOMAS M D A/AU
E5            4      THOMAS M D H/AU
E6            3      THOMAS M D JR/AU
E7            2      THOMAS M D O/AU
E8           14      THOMAS M D R/AU
E9          338      THOMAS M E/AU
E10          33      THOMAS M E A/AU
E11           5      THOMAS M E C/AU
E12          32      THOMAS M E M/AU

=> s e3-e8
L18         518 ("THOMAS M D"/AU OR "THOMAS M D A"/AU OR "THOMAS M D H"/AU OR
                "THOMAS M D JR"/AU OR "THOMAS M D O"/AU OR "THOMAS M D R"/AU)

=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1        377498 S BACILLUS
L2             0 S ALPHA(A)AMYOLASE
L3         52121 S ALPHA(A)AMYLASE
L4         10118 S L1 AND L3
L5            77 S "AMYQ"
L6            57 S L4 AND L5
L7            23 DUP REM L6 (34 DUPLICATES REMOVED)
L8           310 S "TTGACA"
L9             1 S L7 AND L8
L10          428 S "TATAAT"
L11          109 S L8 AND L10
L12           35 S L1 AND L11
L13           15 DUP REM L12 (20 DUPLICATES REMOVED)
L14           26 S L5 AND PROMOTER
L15           13 DUP REM L14 (13 DUPLICATES REMOVED)
                 E WIDNER W/AU
L16          113 S E3-E9
                 E SLOMA A/AU
L17          203 S E3-E7
                 E THOMAS M D/AU
L18          518 S E3-E8

=> s l16 or l17 or l18
L19         795 L16 OR L17 OR L18

=> s l12 and l19
L20           5 L12 AND L19

=> dup rem l20
PROCESSING COMPLETED FOR L20
L21           4 DUP REM L20 (1 DUPLICATE REMOVED)

=> d 1-4 ibib ab

L21   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483  BIOTECHDS
TITLE:            Production of a secreted polypeptide having L-asparaginase
                  activity for treating leukemia, comprises cultivating a host
```

cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy

AUTHOR: **THOMAS M D; SLOMA A**
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]

AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis CryIIIA gene (cryIIIA) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the cryIIIA mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells

for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L21 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: **WIDNER W; SLOMA A; THOMAS M D**
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which

generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L21 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1

ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a **Bacillus** cell.
AUTHOR(S): **Widner, William** [Inventor, Reprint author]; **Sloma, Alan** [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium

conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L21 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S): **Widner, William**; **Sloma, Alan**; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:	US 1998-31442	A	19980226
	US 1999-256377	B3	19990224
	WO 1999-US4360	W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid

sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein

the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

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=> d his

     (FILE 'HOME' ENTERED AT 08:06:44 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 08:07:09 ON 12 MAY 2005
L1       377498 S BACILLUS
L2            0 S ALPHA(A)AMYOLASE
L3        52121 S ALPHA(A)AMYLASE
L4        10118 S L1 AND L3
L5           77 S "AMYQ"
L6           57 S L4 AND L5
L7           23 DUP REM L6 (34 DUPLICATES REMOVED)
L8          310 S "TTGACA"
L9            1 S L7 AND L8
L10         428 S "TATAAT"
L11         109 S L8 AND L10
L12          35 S L1 AND L11
L13          15 DUP REM L12 (20 DUPLICATES REMOVED)
L14          26 S L5 AND PROMOTER
L15          13 DUP REM L14 (13 DUPLICATES REMOVED)
                E WIDNER W/AU
L16         113 S E3-E9
                E SLOMA A/AU
L17         203 S E3-E7
                E THOMAS M D/AU
L18         518 S E3-E8
L19         795 S L16 OR L17 OR L18
L20           5 S L12 AND L19
L21           4 DUP REM L20 (1 DUPLICATE REMOVED)
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	Document ID	Kind Codes	Source	Issue Date	Pages
1	US 20030186380 A1		US-PGPUB	20031002	22
2	US 20030175902 A1		US-PGPUB	20030918	142
3	US 20030170876 A1		US-PGPUB	20030911	57
4	US 6255076 B1		USPAT	20010703	54

	Title
1	Methods for producing secreted polypeptides having L-asparaginase activity
2	Methods for producing hyaluronan in a recombinant host cell
3	Methods for producing a polypeptide in a bacillus cell
4	Methods for producing a polypeptide in a Bacillus cell

	Document ID	Kind Codes	Source	Issue Date	Pages
1	US 20030186380 A1		US-PGPUB	20031002	22
2	US 20030175902 A1		US-PGPUB	20030918	142
3	US 20030170876 A1		US-PGPUB	20030911	57
4	US 6551813 B1		USPAT	20030422	60
5	US 6255076 B1		USPAT	20010703	54
6	US 5171673 A		USPAT	19921215	20

	Title
1	Methods for producing secreted polypeptides having L-asparaginase activity
2	Methods for producing hyaluronan in a recombinant host cell
3	Methods for producing a polypeptide in a bacillus cell
4	Nutrient medium for bacterial strains which overproduce riboflavin
5	Methods for producing a polypeptide in a Bacillus cell
6	Expression of heterologous DNA using the bacillus coagulans amylase gene

	L #	Hits	Search Text
1	L1	0	("bacillus").PN.
2	L2	37056	bacillus
3	L3	404	"TATAAT"
4	L4	165	"TTGACA"
5	L5	449	l3 or l4
6	L6	13	l2 same l5
7	L7	7967	alpha adj amylase$2
8	L8	5	l6 same l7
9	L9	2497	l2 adj stearothermophilus
10	L10	503	l7 same l9
11	L11	1	l6 same l10
12	L12	242	"amyQ"
13	L13	4	l6 same l12
14	L14	386939	WIDNER SLOMA THOMAS
15	L15	6	l6 and l14

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=> d his

     (FILE 'HOME' ENTERED AT 11:19:17 ON 12 MAY 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:19:42 ON 12 MAY 2005
L1        377498 S BACILLUS
L2           310 S "TTGACA"
L3           428 S "TATAAT"
L4           629 S L2 OR L3
L5           115 S L1 AND L4
L6           657 S "MARKER-FREE"
L7          1364 S MARKER (1W)FREE
L8          1364 S L6 OR L7
L9             1 S L5 AND L8
L10           23 S L1 AND L8
L11           12 DUP REM L10 (11 DUPLICATES REMOVED)
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NEWS	13	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags
NEWS	14	APR 04	EPFULL enhanced with additional patent information and new fields
NEWS	15	APR 04	EMBASE - Database reloaded and enhanced
NEWS	16	APR 18	New CAS Information Use Policies available online
NEWS	17	APR 25	Patent searching, including current-awareness alerts (SDIs), based on application date in CA/CAplus and USPATFULL/USPAT2 may be affected by a change in filing date for U.S. applications.
NEWS	18	APR 28	Improved searching of U.S. Patent Classifications for U.S. patent records in CA/CAplus

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=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

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FILE 'LIFESCI' ENTERED AT 11:19:42 ON 12 MAY 2005
COPYRIGHT (C) 2005 Cambridge Scientific Abstracts (CSA)


=> s bacillus
L1      377498 BACILLUS

=> s "TTGACA"
L2         310 "TTGACA"

=> s "TATAAT"
L3         428 "TATAAT"

=> s l2 or l3
L4         629 L2 OR L3

=> s l1 and l4
L5         115 L1 AND L4

=> s "marker-free"
L6         657 "MARKER-FREE"

=> s marker (1w)free
L7        1364 MARKER (1W) FREE

=> s l6 or l7
L8        1364 L6 OR L7

=> s l5 and l8
L9           1 L5 AND L8

=> d all
```

L9 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
AN 2004-04169 BIOTECHDS
TI Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AU WIDNER W; SLOMA A; THOMAS M D
PA NOVOZYMES BIOTECH INC
PI US 2003170876 11 Sep 2003
AI US 2001-834271 12 Apr 2001
PRAI US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DT Patent
LA English
OS WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:
NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable **marker-free** mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable **marker-free** mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase,

	Document ID	Kind Codes	Source	Issue Date	Pages
1	US 20030170876 A1		US-PGPUB	20030911	57
2	US 6255076 B1		USPAT	20010703	54
3	US 5955310 A		USPAT	19990921	39

	Title
1	Methods for producing a polypeptide in a bacillus cell
2	Methods for producing a polypeptide in a Bacillus cell
3	Methods for producing a polypeptide in a bacillus cell

	L #	Hits	Search Text
1	L1	37056	bacillus
2	L2	165	"TTGACA"
3	L3	404	"TATAAT"
4	L4	449	l2 or l3
5	L5	13	l1 same l4
6	L6	182	marker adj free
7	L7	1	l4 same l6
8	L8	3	l1 same l6

cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable **marker-free** mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

CC .THERAPEUTICS, Protein Therapeutics; GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis

CT RECOMBINANT PROTEIN PREP., ISOL., VECTOR-MEDIATED GENE TRANSFER, EXPRESSION IN HOST CELL, APPL. **BACILLUS** SP. SELECTABLE MARKER BACTERIUM (23, 08)

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:19:42 ON 12 MAY 2005
L1        377498 S BACILLUS
L2           310 S "TTGACA"
L3           428 S "TATAAT"
L4           629 S L2 OR L3
L5           115 S L1 AND L4
L6           657 S "MARKER-FREE"
L7          1364 S MARKER (1W)FREE
L8          1364 S L6 OR L7
L9             1 S L5 AND L8

=> s l1 and l8
L10           23 L1 AND L8

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           12 DUP REM L10 (11 DUPLICATES REMOVED)

=> d 1-12 ibib ab
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L11 ANSWER 1 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-06683 BIOTECHDS
TITLE: New integrative method to generate **Bacillus** subtilis recombinant strains free of selection markers; recombinant selectable **marker-free** bacterium production via plasmid expression in host cell using auxotrophy method
AUTHOR: BRANS A; FILEE P; CHEVIGNE A; CLAESSENS A; JORIS B
CORPORATE SOURCE: Univ Liege
LOCATION: Joris B, Univ Liege, Inst Chem B6a, Ctr Prot Engn, B-4000 Liege, Belgium
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY; (2004) 70, 12, 7241-7250
ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - The novel method described in this paper combines the use of blaI, which encodes a repressor involved in **Bacillus** licheniformis BlaP beta-lactThetaamase regulation, an antibiotic resistance gene, and a B. subtilis strain (BS1541) that is conditionally auxotrophic for lysine. We constructed a BlaI cassette containing blaI and the spectinomycin resistance genes and two short direct repeat DNA sequences, one at each extremity of the cassette. The BS1541 strain was obtained by replacing the B. subtilis P-lysA promoter with that of the P-blaP beta-lactamase promoter. In the resulting strain, the cloning of the blaI repressor gene confers lysine auxotrophy to BS1541. After integration of the Mal cassette into the chromosome of a conditionally lys-auxotrophic (BS1541) strain by homologous recombination and positive selection for spectinomycin resistance, the eviction of the Mal cassette was achieved by single crossover between the two short direct repeat sequences. This strategy was successfully used to inactivate a single gene and to introduce a gene of interest in the **Bacillus** chromosome. In both cases the resulting strains are free of selection marker. This allows the use of the BlaI cassette to repeatedly further modify the **Bacillus** chromosome. (10 pages)

L11 ANSWER 2 OF 12 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:25846 SCISEARCH
THE GENUINE ARTICLE: 880OA
TITLE: Cre/lox-mediated marker gene excision in elite indica rice plants transformed with genes conferring resistance to lepidopteran insects
AUTHOR: Chen S B; Liu X; Peng H Y; Gong W K; Wang R; Wang F; Zhu Z (Reprint)
CORPORATE SOURCE: Chinese Acad Sci, Inst Genet & Dev Biol, Beijing 100101, Peoples R China (Reprint); Fujian Acad Agr Sci, Fujian Provincial Key Lab Agr Genet Engn, Fuzhou 350003, Peoples R China
COUNTRY OF AUTHOR: Peoples R China
SOURCE: ACTA BOTANICA SINICA, (DEC 2004) Vol. 46, No. 12, pp. 1416-1423.
Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA.
ISSN: 1672-6650.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 44
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Cre/lox-mediated gene excision in commercial rice (Oryza sativa L.) plants was studied with a recombination-reporter gene system, in which the selectable marker hygromycin phosphotransferase gene (hpt) flanking by two

directly oriented lox sites was located between the rice actin 1 promoter and a promoterless gusA gene. This system allows visualization of GUS expression by activating promoterless gusA after site-specific recombination. The crossing strategy was used to introduce the cre gene into the lox plants. In 30 hybrid plants from four crosses made from T-0 actin1 promoter-lox-hpt-lox-gusA plant with T-0 cre plant, 12 expressed GUS and 9 showed hygromycin-sensitive. We furthermore demonstrated the utility of the Cre/lox in excision of hpt marker gene in an elite indica rice restorer Minghui 86 transformed with both insecticidal modified cowpea trypsin inhibitor gene sck and **Bacillus** thuringiensis endotoxin gene cryIAc. In 77 hybrid plants from nine crosses made from T-2 homozygous lox-hpt-lox-sck-cryIAc plant with T-2 homozygous cre plant, 56 showed hygromycin-sensitive. Molecular analyses confirmed the excision of hpt in all hygromycin-sensitive plants.

L11 ANSWER 3 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:
NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable **marker-free** mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable **marker-free** mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the

tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable **marker-free** mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L11 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 1

ACCESSION NUMBER: 2003-05448 BIOTECHDS
TITLE: New plasmid vector, useful for genomic modification of coryneform bacteria, is non-replicable in host cells and includes the sacB gene as negative-dominant marker; recombinant vector plasmid-mediated gene transfer and expression in Corynebacterium glutamicum or Escherichia coli for use in pharmaceutical, agricultural and cosmetic industry
AUTHOR: POMPEJUS M; KROEGER B; SCHROEDER H; ZELDER O
PATENT ASSIGNEE: BASF AG
PATENT INFO: DE 10109996 5 Sep 2002
APPLICATION INFO: DE 2001-1009996 1 Mar 2001
PRIORITY INFO: DE 2001-1009996 1 Mar 2001; DE 2001-1009996 1 Mar 2001
DOCUMENT TYPE: Patent

LANGUAGE: German
OTHER SOURCE: WPI: 2003-047838 [05]
AB DERWENT ABSTRACT:
NOVELTY - Plasmid vector (A) that is not replicable in target organisms comprising an origin of replication for Escherichia coli, at least one genetic marker, optionally a sequence (mob) that permits transfer of DNA by conjugation, segment (B) that is homologous to a segment in the target and mediates homologous recombination, and the sacB gene from **Bacillus** amyloliqufaciens, under control of a promoter, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) **marker-free** mutagenesis in Gram-positive bacteria, using (A); and(2) bacteria produced by method (1).

BIOTECHNOLOGY - Preferred Plasmid: The marker imparts antibiotic resistance (to kanamycin, chloramphenicol, tetracycline or ampicillin), the promoter is heterologous (from E. coli or corynebacterium glutamicum, specifically tac), and the mob sequence is present. Preferred Process: In method (1), (A) is transferred into a Gram-positive bacterium and selection made for at least one genetic marker. Clones are then selected from the transformants by growing them on sucrose-containing medium. The bacteria are particularly from the genera Brevibacterium and Corynebacterium and transfection is by electroporation or conjugation.

USE - (A) is used to introduce genomic modifications (deletions, disruptions, (multiple) point mutations or complete gene exchanges) into Brevibacterium and Corynebacterium, which are used for production of fine chemicals (e.g. fatty, amino or other acids, nucleosides, nucleotides, lipids etc., for use in pharmaceuticals, agriculture and cosmetics), also for degradation of hydrocarbons and oxidation of terpenoids. Most preferably the genetic modification leads to reduced formation of by-products during fermentation, especially it promotes formation of a desired product or overcomes a bottleneck.

ADVANTAGE - sacB is particularly well suited as conditional, dominant-negative marker gene for coryneforms.

EXAMPLE - To inactivate the ddh gene in Corynebacterium glutamicum, segments from the 5'- and 3'-ends of the gene were amplified by polymerase chain reaction, then ligated to form an inactive gene. This product and the sacB gene (for levan sucrase) from **Bacillus** amyloliqufaciens, under control of the trc promoter, were cloned into pSL18 (J. Microbiol. Biotechnol., 6 (1996) 315) to produce pSL18sacBDELTAddh. The recombinant plasmid was introduced into C. glutamicum by conjugation or electroporation and integrants selected on kanamyin-containing medium. Selected colonies were then plated on to sucrose-containing and sucrose-free media and those that express sacB selected from growth on the sucrose-free plates only. They have an inactivated ddh gene. (12 pages)

L11 ANSWER 5 OF 12 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:778171 HCAPLUS
DOCUMENT NUMBER: 137:289892
TITLE: Antibiotic-free bacterial strain selection with antisense molecules against essential genes
INVENTOR(S): Nielsen, Peter E.; Good, Liam
PATENT ASSIGNEE(S): Kobenhavns Universitet, Den.
SOURCE: PCT Int. Appl., 92 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002079467	A2	20021010	WO 2002-DK208	20020326

WO 2002079467 A3 20040304
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: DK 2001-523 A 20010329

AB A new method for an antibiotic-free selection of genetically modified cells is described. It is shown that antisense mols. targeted to an essential gene inhibit growth may be used for growth selection of cells transformed with a plasmid carrying an altered version of the essential gene. The antisense mol. may be an antisense DNA or an antisense peptide nucleic acid (PNA). The results show that antisense mols. may be used for antibiotic-free selection of desired transformed microbes when targeted against an essential microbial gene. This technol. is useful in genetic engineering for research growth and isolation of transformed organisms, and for industrial growth maintenance of transformed organisms, e.g. in the production of genetically engineered proteins as an environmentally safer alternative to traditional selection methods based on antibiotics. Antisense peptide nucleic acids are included in the incubation medium in the same way as an antibiotic would be used. Preliminary optimization expts. used antisense PNA to the lacZ gene. These expts. found the optimum length range for effective inhibition of gene expression and the effects of potential carrier peptides on the bactericidal activity of the PNAs. Use of antisense PNA to the acyl-carrier protein gene acpP of Escherichia coli and of **Bacillus** subtilis as a selectable marker is demonstrated. The use of antisense PNA to inhibit expression of a reporter gene without adverse effects on the host.

L11 ANSWER 6 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
ACCESSION NUMBER: 2002:564731 BIOSIS
DOCUMENT NUMBER: PREV200200564731
TITLE: Transposon-mediated generation of T-DNA- and **marker -free** rice plants expressing a Bt endotoxin gene.
AUTHOR(S): Cotsaftis, Olivier; Sallaud, Christophe; Breitler, Jean Christophe; Meynard, Donaldo; Greco, Rafaella; Pereira, Andy; Guiderdoni, Emmanuel [Reprint author]
CORPORATE SOURCE: Biotrop Programme, Cirad-Amis, TA40/03, F-34398, Montpellier Cedex 5, France
guiderdoni@cirad.fr
SOURCE: Molecular Breeding, (2002) Vol. 10, No. 3, pp. 165-180. print.
ISSN: 1380-3743.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Nov 2002
Last Updated on STN: 7 Nov 2002

AB Transposon-mediated repositioning of transgenes is an attractive strategy to generate plants that are free of selectable markers and T-DNA inserts. By using a minimal number of transformation events a large number of transgene insertions in the genome can be obtained so as to benefit from position effects in the genome that can contribute to higher levels of expression. We constructed a **Bacillus** thuringiensis synthetic cry1B gene expressed under control of the maize ubiquitin promoter between minimal terminal inverted repeats of the maize Ac-Ds transposon system, which was cloned in the 5' untranslated sequence of a gfp gene used as an

excision marker. The T-DNA also harboured the Ac transposase gene driven by the CaMV 35S promoter and the hph gene conferring resistance to the antibiotic hygromycin. Sixty-eight independent rice (Oryza sativa L.) transformants were regenerated and molecularly analysed revealing excision and reinsertion of the Ds-cry1B element in 37% and 25% respectively of the transformation events. Five independent transformants harbouring 2-4 reinserted Ds-Cry1B copies were analysed in the T1 progeny, revealing 0.2 to 1.4 new transpositions per plant. Out segregation of the cry1B gene from the T-DNA insertion site was observed in 17 T1 plants, representing 10 independent repositioning events without selection. Western analysis of leaf protein extracts of these plants revealed detectable Cry1B in all the plants indicating efficient expression of the transgene reinsertions. Stability of position and expression of the cry1B transgene was further confirmed in T2 progeny of T-DNA-free T1 plants. New T-DNA-free repositioning events were also identified in T2 progenies of T1 plants heterozygous for the T-DNA. Furthermore, preliminary whole plant bioassay of T-DNA-free lines challenged with striped stem borer larvae suggested that they are protected against SSB attacks. These results indicate that transposon mediated relocation of the gene of interest is a powerful method for generating T-DNA integration site-free transgenic plants and exploiting favourable position effects in the plant genome.

L11 ANSWER 7 OF 12 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3

ACCESSION NUMBER: 2001030732 EMBASE
TITLE: Development of **marker-free** strains of **Bacillus** subtilis capable of secreting high levels of industrial enzymes.
AUTHOR: Widner B.; Thomas M.; Sternberg D.; Lammon D.; Behr R.; Sloma A.
CORPORATE SOURCE: Dr. B. Widner, Novo Nordisk Biotech., Inc., Davis, CA 95616, United States. wwidner@nnbt.com
SOURCE: Journal of Industrial Microbiology and Biotechnology, (2000) Vol. 25, No. 4, pp. 204-212.
Refs: 31
ISSN: 1367-5435 CODEN: JIMBFL
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20010208
Last Updated on STN: 20010208

AB Different strategies have been employed to achieve high-level expression of single-copy genes encoding secreted enzymes in **Bacillus** subtilis. A model system was developed which utilizes the aprL gene from **Bacillus** clausii as a reporter gene for monitoring expression levels during stationary phase. An exceptionally strong promoter was constructed by altering the nuceotide sequence in the -10 and -35 regions of the promoter for the amyQ gene of **Bacillus** amyloliquefaciens. In addition, two or three tandem copies of this promoter were shown to increase expression levels substantially in comparison to the monomer promoter alone. Finally, the promoter and mRNA stabilization sequences derived from the cry3A gene of **Bacillus** thuringiensis were used in combination with the mutant amyQ promoter to achieve the highest levels of aprL expression. These promoters were shown to be fully functional in a high-expressing **Bacillus** strain grown under industrial fermentation conditions. The ability to obtain maximum expression levels from a single copy gene now makes it feasible to construct environmentally friendly, **marker-free** industrial strains of B. subtilis.

L11 ANSWER 8 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1999-15556 BIOTECHDS
TITLE: Production of polypeptide in **Bacillus** sp. using specific promoters, particularly for producing enzymes; the effect of a short consensus amyQ promoter on recombinant alpha-amylase production via vector-mediated gene transfer and expression in **Bacillus** subtilis
AUTHOR: Widner W; Sloma A; Thomas M D
PATENT ASSIGNEE: Novo-Nordisk-Biotech
LOCATION: Davis, CA, USA.
PATENT INFO: WO 9943835 2 Sep 1999
APPLICATION INFO: WO 1999-US4360 26 Feb 1999
PRIORITY INFO: US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-561370 [47]
AB The production of a protein (I) in **Bacillus** sp. cells using specific tandem or consensus promoters is new. Also claimed are: the production of the recombinant **Bacillus** sp. cells via the introduction of a nucleic acid construct; the production of **Bacillus** sp. mutants which contain no selectable marker genes by treating the cells to delete a marker gene; **marker-free** mutant cell produced using this method; isolated consensus alpha-amylase (amyQ) promoter sequence made up of 2 185 bp DNA sequences (specified); a nucleic acid construct containing a sequence (II), which encodes (I), linked to one or more copies of the amyQ promoter; and a recombinant vector and **Bacillus** sp. cells containing this construct. This new method may be useful for producing homologs or particularly heterologous proteins, particularly enzymes (specifically serine protease, maltogenic alpha-amylase, EC-3.2.1.1 and pullulanase, EC-3.2.1.41), but also hormones, antibodies, reporters, etc. In an example, the replacement of the amyQ promoter with a short consensus amyQ promoter resulted in a increase in enzyme expression of 620% in **Bacillus** subtilis strain PL801 cells. (89pp)

L11 ANSWER 9 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1997-05933 BIOTECHDS
TITLE: Stable maintenance of a transformed plasmid in a host cell; using a plasmid with an operator, and chromosomal repressor and operator-associated essential gene, for improved plasmid stability; recombinant protein expression and gene therapy
AUTHOR: Sherratt D J; Williams S G; Hanak J A J
PATENT ASSIGNEE: Therexsys
LOCATION: Keele, UK.
PATENT INFO: WO 9709435 13 Mar 1997
APPLICATION INFO: WO 1996-GB2208 6 Sep 1996
PRIORITY INFO: GB 1995-18395 8 Sep 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-192910 [17]
AB A new **marker-free** recombinant host cell contains: a plasmid with an operator which binds a repressor; a 1st chromosomal gene encoding the repressor; and a 2nd chromosomal gene functionally associated with the operator, essential for cell growth. The plasmid is present in sufficient numbers to titrate the repressor so that the essential gene is expressed, permitting cell growth. The repressor is preferably the Escherichia coli lac, gamma, trp, galR, araC, tet or ArgRNV repressor. The host is preferably a mammal, insect, plant, fungus, yeast or bacterium cell, e.g. E. coli, Salmonella sp. or **Bacillus** sp. The plasmid may be 1,000 bp in size, and may have a replication origin allowing replication of 10-50 copies/cell (e.g. plasmid pBR322) or 100-200 copies/cell (e.g. plasmid pUC), a cloning

site, and a target gene operatively associated with mammal cell control sequences. A new method for maintaining a plasmid in a host cell and recombinant protein production involves culture of a recombinant cell containing the new vector and allowing growth to occur. The plasmid may be used in gene therapy, or may encode a therapeutic protein. (48pp)

L11 ANSWER 10 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-12115 BIOTECHDS
TITLE: New DNA constructs associated with a transposase gene; gene expression in selectable **marker free** e.g. **Bacillus** subtilis, **Bacillus** licheniformis, **Bacillus** brevis, etc. and Lactobacillus sp., for expression of a desired DNA sequence
AUTHOR: Jorgensen S T
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 9623073 1 Aug 1996
APPLICATION INFO: WO 1996-DK38 23 Jan 1996
PRIORITY INFO: DK 1995-799 6 Jul 1995; DK 1995-83 23 Jan 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1996-362695 [36]
AB The following are claimed: 1) DNA constructs (of specified DNA sequence) and which are associated with a transposase gene T which is located on either side of and outside the structure, and are composed of transposase target sequences, a DNA sequence of interest, a target sequence for a site-specific recombination enzyme, and a selectable marker gene; 2) a vector comprising any of the above DNA constructs; 3) a bacterial cells, which in it's constitutive DNA has integrated at least 2 copies of a DNA construct as above; and 4) a **marker-free** cell of a Gram-positive bacterium comprising multiple copies of a DNA sequence of interest, and which is selected from Lactobacillus sp. or **Bacillus** subtilis, **Bacillus** licheniformis, **Bacillus** lentus, **Bacillus** brevis, **Bacillus** stearothermophilus, **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** coagulans, **Bacillus** circulans, **Bacillus** lautus, **Bacillus** megaterium, and **Bacillus** thuringiensis. The DNA constructs are used for constructing bacterial cells in which their genomic DNA have integrated at least 1 copy of a DNA sequence of interest, free from an undesired selectable marker. (143pp)

L11 ANSWER 11 OF 12 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4
ACCESSION NUMBER: 96:173876 SCISEARCH
THE GENUINE ARTICLE: TX259
TITLE: CONDITIONALLY REPLICATIVE AND CONJUGATIVE PLASMIDS CARRYING LACZ-ALPHA FOR CLONING, MUTAGENESIS, AND ALLELE REPLACEMENT IN BACTERIA
AUTHOR: METCALF W W; JIANG W H; DANIELS L L; KIM S K; HALDIMANN A; WANNER B L (Reprint)
CORPORATE SOURCE: PURDUE UNIV, DEPT BIOL SCI, W LAFAYETTE, IN, 47907 (Reprint); PURDUE UNIV, DEPT BIOL SCI, W LAFAYETTE, IN, 47907
COUNTRY OF AUTHOR: USA
SOURCE: PLASMID, (JAN 1996) Vol. 35, No. 1, pp. 1-13. ISSN: 0147-619X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 26
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We describe several new cloning vectors for mutagenesis and allele replacement experiments. These plasmids have the R6K gamma DNA replication origin (oriR(R6K gamma)) so they replicate only in bacteria supplying the Pi replication protein (encoded by pir), and they can be maintained at low or high plasmid copy number by using Escherichia coli strains encoding either wild-type or mutant forms of Pi. They also carry the RP4 transfer origin (oriT(RP4)) SO they can be transferred by conjugation to a broad range of bacteria. Most of them encode lacZ alpha for blue-white color screening of colonies for ones with plasmids carrying inserts, as well as the f1 DNA replication origin for preparation of single-stranded DNA. Particular plasmids are especially useful for allele replacement experiments because they also encode a positive counterselectable marker. One set carries tetAR (from Tn10) that allows for positive selection of plasmid-free segregants as tetracycline-sensitive (Tet(S)) recombinants. Another set carries sacB (from **Bacillus** subtilis) that allows selecting plasmid-free segregants as sucrose-resistant (Suc(R)) ones. Accordingly, derivatives of these plasmids can be introduced into a non-pir host (via conjugative transfer, transformation, or electroporation), and integrants with the plasmid recombined into the chromosome via homologous sequences are selected using a plasmid antibiotic resistance **marker**. Plasmid-**free** segregants with an allele replacement can be subsequently selected as Tet(S) or Suc(R) recombinants. A number of additional features (including the presence of multiple cloning sites flanked by T3 and T7 RNA polymerase promoters) make these plasmids useful as general cloning vectors as well. (C) 1996 Academic Press, Inc.

L11 ANSWER 12 OF 12 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 5
ACCESSION NUMBER: 1995-04246 BIOTECHDS
TITLE: Selection **marker** gene **free** recombinant strains, especially filamentous fungi, and methods for obtaining them;
acetamidase selectable marker deletion on integration of a chymosin, phytase, endo-1,4-beta-D-xylanase, lipase, amylase, protease or beta-galactosidase gene
AUTHOR: Selten G C M; van Gorcom R F M; Swinkels B W
PATENT ASSIGNEE: Brocades
PATENT INFO: EP 635574 25 Jan 1995
APPLICATION INFO: EP 1994-201896 30 Jun 1994
PRIORITY INFO: EP 1993-202195 23 Jul 1993
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1995-053686 [08]
AB A host transformed 1 or more vector(s) contains target DNA (integrated into the genome via site-specific homologous recombination) and a deleted selectable marker (SM, e.g. Aspergillus sp. acetamidase) gene. The target DNA contains a chymosin (EC-3.4.23.4), phytase, endo-1,4-beta-D-xylanase (EC-3.2.1.8), lipase (EC-3.1.1.3), amylase, protease or beta-galactosidase (EC-3.2.1.23) gene, cDNA, promoter, terminator, regulatory element, intron, DNA binding protein recognition site, translation initiation site and/or restriction site. The host is Aspergillus, Trichoderma or Penicillium (preferred strains), Kluyveromyces, Saccharomyces, **Bacillus** licheniformis, **Bacillus** subtilis or Escherichia coli. At least 2 mutations may be introduced using the SM, with deletion resulting from target DNA integration. An SM-free strain free of undesired DNA for use in food or pharmaceutical production may be obtained by: integration of target DNA and a dominant bidirectional SM into the genome; selection; SM deletion by recombination between flanking repeats; and counter-selection for absence of the SM. (109pp)

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 11:19:42 ON 12 MAY 2005
L1        377498 S BACILLUS
L2           310 S "TTGACA"
L3           428 S "TATAAT"
L4           629 S L2 OR L3
L5           115 S L1 AND L4
L6           657 S "MARKER-FREE"
L7          1364 S MARKER (1W)FREE
L8          1364 S L6 OR L7
L9             1 S L5 AND L8
L10           23 S L1 AND L8
L11           12 DUP REM L10 (11 DUPLICATES REMOVED)
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